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ELECTRON PARAMAGNETIC RESONANCE STUDIES OF GAMMA-IRRADIATED POLYPEPTIDES

BY

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ELECTRON PARAMAGNETIC RESONANCE
STUDIES OF γ-IRRADIATED POLYPEPTIDES

by

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A dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of
Philosophy in the Department of Physics
in the Graduate School of Arts and
Sciences of Duke University

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ELECTRON PARAMAGNETIC RESONANCE STUDIES OF γ-IRRADIATED POLYPEPTIDES

Chapter I

INTRODUCTION

Electron paramagnetic resonance studies began in 1945 with the pioneering work of Zavoisky[1] in Russia. Stimulated by the early work of Gorter[2] in the field of paramagnetic relaxation, Zavoisky detected the first electron resonance in solutions containing paramagnetic ions. Subsequent improvements in detection efficiency due to the application of more advanced microwave techniques soon led to the observation of this spin-resonance phenomenon in solids and gasses as well.

In electron spin resonance, magnetic dipole transitions are observed between energy levels of a system which is perturbed by a large magnetic field, such that the transitions take place in the microwave region. The basic theory governing such transitions was well known; nevertheless, further developments in this field resulted in the formulation of the 'spin Hamiltonian' treatment of Abragam and Price,[3] and then application of molecular orbital theory and valence bond theory, with the additional refinements of configurational interaction and hyperconjugation, in order to explain the complex hyperfine structure observed in many spin-resonance spectra. Thus, electron paramagnetic resonance had established itself as a valuable tool

for the study of inorganic materials when, in 1954, Commoner, Townsend, and Pake[4] first observed the paramagnetic resonance of naturally occurring unpaired electrons in biological substances. This discovery was soon followed by the application of electron paramagnetic resonance techniques to the investigation of a wide variety of organic preparations of biological significance.

These experiments may be broadly grouped into two main classes: (a) Those involving naturally occurring unpaired electrons[5, 6, 7] and (b) those involving free radicals[*] produced as intermediates in biological chemical reactions,[9, 10] or artificially created by incident-ionizing radiation. In the latter field, the advent of nuclear explosions and readily available high energy sources of ionizing radiation accelerated interest in the mechanisms and effects of radiation damage in biological material.

Extensive studies of irradiated proteins,[11, 12] amino acids,[13] and associated biological systems[14] have been undertaken in this laboratory. Ordinarily these substances do not possess unpaired electrons and therefore would not exhibit paramagnetic resonance absorption; however, it has been found that exposure to radiation with sufficient energy to ionize the molecule or break molecular bonds will in many cases result in the formation of free radicals with long lifetimes, often of the order of months.[15] Since one of the characteristics of a free radical, due to the presence of an unpaired electron in the outer orbitals of the system, is an extremely high chemical reactivity, this long lifetime would indicate some type of trapping mechanism (the cage effect) which inhibits changes in the radicals formed. Further discussion of

---

[*] A free radical may be defined as[8] 'a molecule, or part of a molecule, in which the normal chemical binding has been modified so that an unpaired electron is left associated with the system.'

this effect will be found in Chapter II.

Studies of the formation of free radicals in proteins and amino acids at 77°K and the subsequent changes in these radicals as the samples are warmed to 300°K, have led to the conclusion that, at first, primary radicals are formed in these compounds which then change when warmed to other secondary forms.[16] The results of this work indicated that γ-irradiated proteins give characteristically different electron paramagnetic resonance patterns when irradiated and observed at 77°K, and the possibility of using electron paramagnetic resonance coupled with ionizing irradiation was proposed as a new method for identification of the amino acid residue in proteins.

Early work with the simple di-peptide glycyl-glycine[17] and several other di-peptides and tri-peptides[18] had shown that the paramagnetic resonance spectrum of an amino acid was altered when this amino acid becomes part of a longer chain molecule through formation of the peptide bond, and that effects of the terminal groups are often important in determining the type of radical formed for these short chain molecules. Identification of radicals characteristic of the particular amino acid residues would then require investigation of fairly long polypeptides artificially created from only one amino acid. Recently, these have become commercially available as a result of rapid advances in a one-step polymerization process as reported by Katchalski.[19]

These preliminary developments immediately suggested the investigations which are the subject of this thesis. Eighteen of these synthetic polypeptides, composed of only one type of amino acid residue, have been studied,

---

* Polypeptides used in these studies may be purchased from the Schwartz Biochemical Research Co., Mt. Vernon, N. Y.

and a discussion of the results obtained will be found in Chapter IV. The paramagnetic resonance spectrum was observed for each substance under the following conditions: (a) Irradiated and observed in vacuum at 300°K, (b) irradiated and observed in vacuum at 77°K, (c) warming from 77°K to 300°K, (d) opened to air at 300°K. A complete compilation of the spectra observed in (a) and (b) above is included, as well as representative tracings of the effects noted in (c) and (d) for various samples.

Chapter II

THEORY

A. The Resonance Condition. One of the characteristics of free radicals and paramagnetic atoms or ions is the presence of an unpaired electron, that is, one whose angular momentum is not cancelled out by other surrounding electrons, and which, therefore, possesses a magnetic dipole moment, $\vec{\mu}$. The magnetic moment may be related to the spin and orbital angular momenta of the electron, designated by $\vec{s}$ and $\vec{\ell}$, in the following manner:

$$\vec{\mu} = -g_s \beta \vec{s} - g_\ell \beta \vec{\ell} \qquad (1)$$

where β is the usual Bohr magneton, given by $\frac{e\hbar}{2mc}$, and g_s and g_ℓ are factors which indicate the relative contributions of the spin and orbital angular momenta to the magnetic moment.

The energy of interaction of the magnetic dipole with an external magnetic field, $\vec{H}$, is given by

$$W = -\vec{\mu} \cdot \vec{H} \qquad (2)$$

For weak fields, $\vec{\ell}$ and $\vec{s}$ combine vectorially to give a resultant total angular momentum, $\vec{j}$, which precesses about the direction of the field.

The component of magnetic moment along $\vec{j}$ is

$$\mu_j = g\beta j \tag{3}$$

where here g is given by the usual Landé relation, ie.

$$g = 1 + \frac{j(j+1) + s(s+1) - \ell(\ell+1)}{2j(j+1)} \tag{4}$$

The time average of $\vec{\mu}$ perpendicular to $\vec{j}$ is zero. Hence, combining equations 2 and 3, we have

$$W = g\beta \vec{j} \cdot \vec{H} = g\beta m_j H \tag{5}$$

where now m_j is the component of $\vec{j}$ along $\vec{H}$, which may take the values $j, j-1, \cdot \cdot \cdot -j+1, -j$. Thus, we have a series of $2j+1$ energy levels possible for a particular j. Using Bohr's frequency rule

$$h\nu_{1,2} = \Delta E_{1,2} \tag{6}$$

we have

$$h\nu_{m,m'} = g\beta H(\Delta m) \tag{7}$$

This is the resonance condition, relating the frequency of the transition, $\nu_{m,m'}$ between two perturbed Zeeman sublevels and the perturbing field.

Selection rules arise governing Δm from an examination of the probability of transition (in $\sec^{-1}$) between two levels, m and m′, by absorption of electromagnetic radiation. This is given by:

$$P_{m,m'} = \frac{8\pi^3}{h^2} \left| \langle m | \vec{\mu} | m' \rangle \right|^2 \rho(\nu_{m,m'}) \tag{8}$$

where $\rho(\nu_{m,m'})$ is the incident density of radiative energy/unit frequency range, at the resonant frequency, $\nu_{m,m'}$. By using appropriate electronic wave functions, the non-vanishing matrix elements in equation 8 may be calculated. The following conditions on Δm result:

(a) $\Delta m = 0$, for radiation linearly polarized parallel to $\vec{H}$ (corresponds to no transition).

(b) $\Delta m = \pm 1$, for radiation linearly polarized perpendicular to $\vec{H}$.

Therefore, equation 7 becomes simply:

$$h\nu = g\beta H \tag{9}$$

showing that transitions may be induced between the levels for which $\Delta m = \pm 1$, by an oscillating microwave magnetic field of frequency, ν, perpendicular to an external field, $\vec{H}$.

However, since induced emission and induced absorption are both equally probable, a net absorption will occur only if a population difference exists in favor of the lower state. If a system is in thermal equilibrium, the number of atoms in the state m is given by

$$N_m = N_{m+1} e^{\frac{\Delta E(m, m+1)}{kT}} \tag{10}$$

or, for this case, since $\frac{g\beta H}{kT} \ll 1$,

the excess number of atoms in the lower state is:

$$excess = N_m - N_{m+1} \cong N_m\left(\frac{g\beta H}{kT}\right) \tag{11}$$

Now,

$$\left(\text{Power absorbed}\right) = \left(\begin{array}{c}\text{Net excess in}\\ \text{lower state}\end{array}\right) \times \left(\begin{array}{l}\text{Energy}\\ \text{Absorbed/Transition}\end{array}\right) \times \left(\begin{array}{l}\text{Transition}\\ \text{Probability (sec}^{-1}\text{)}\end{array}\right) \tag{12}$$

or

$$\left(\text{Power absorbed}\right) \cong N_m \frac{(g\beta H)^2}{kT} P_{m,m+1} \tag{13}$$

Hence, for greater ease in detection, use of high fields and low temperatures is desirable.

When an unpaired electron absorbs power and goes to a higher level, there must be a mechanism whereby it may lose energy (other than by induced emission) or the populations in the two levels involved would soon become equal, and no further absorption would occur. Relaxation processes account

for this energy loss. Such processes will tend to reduce the lifetime, Δt, in a given state, however, and cause the resonance to be broadened by an amount

$$\Delta E \approx \hbar / \Delta t \qquad (14)$$

according to the uncertainty principle.

Further aspects of line broadening will be discussed in the following sections, while detailed accounts of the effects of saturation and relaxation processes may be found in the literature.[20, 21]

B. The g-factor. Nearly all free radical spectra occur at g-values very close to the free spin g-factor of 2.0023 (variation from 2.0 occurs from a consideration of relativistic effects). This would suggest that the contribution of the orbital angular momentum to the resultant magnetic moment is slight, or an effective quenching of orbital angular momentum has occurred.

For an unpaired electron in a free radical, this effect may be explained by a consideration of the covalent bonding of the atoms in the radical. Delocalization of the unpaired electron onto the surrounding bonded atoms occurs with a resultant quantization of the orbital angular momentum along the axis of the bond. This is essentially a Stark splitting of the $2\ell+1$ degenerate atomic orbital levels by a strong electric field along the molecular bond axis, leaving one orbital level considerably lower in energy than the others. For most cases, this splitting is so large that only the lower orbital level is populated at normal temperatures. Effectively, the system is now an orbital singlet, and an effective orbital angular momentum, $\ell' = 0$, may be assigned.

The internal field interacts directly with the orbital angular momentum, orienting it along the field axis, but only indirectly with the spin angular momentum, through the mechanism of spin-orbit coupling. The perturbing effect of spin-orbit coupling causes the spin to process about the resultant of the

applied magnetic field and the smaller orbital magnetic dipole field, bringing about an asymmetry in the total angular momentum, and consequently in the g-factor. The most pronounced effect of this nature is observed in single crystals,[22, 23] in which the spatial orientation of the molecular bond about which the orbital momentum processes is fixed in the solid. If $g_{\parallel}$ and $g_{\perp}$ are the g-factors observed when the bond axis is parallel and perpendicular to the applied field, the g-factor for the molecular symmetry axis at an arbitrary angle, θ, with respect to the applied field is given by

$$g(\theta)=\left(g_{\parallel}^2\cos^2\theta+g_{\perp}^2\sin^2\theta\right)^{1/2}. \qquad (15)$$

$g_{\parallel}$ and $g_{\perp}$ will be functions of the energy separation of the orbital levels and the magnitude of the spin-orbit coupling.

In the polycrystalline (powder) samples used in these studies, anisotropy in the g-factor appears as a broadening of the lines, due to the random orientations of the individual crystals in the sample.

C. Hyperfine Structure. The great usefulness of electron spin resonance in free radical studies arises from the existence of well resolved and characteristic hyperfine structure. The hyperfine splitting of an electron resonance line occurs because of the interaction between the magnetic moment of the unpaired electron and the magnetic moment of nuclei which are included in the molecular orbital of the electron. Abragam and Price[24] have developed a theory for such an interaction for the particular case of a single nucleus in the electron paramagnetic resonance of hydrated iron-group crystals, which is sufficiently general for application to free radicals. In this work, a 'spin Hamiltonian' is derived, in which a series of terms describing each interaction is included. For free radicals, assuming orbital quenching, only two terms are important:

$$\mathcal{H}=g_{ij}\beta H_i S_j+A_{ij}S_i I_j\,. \qquad (16)$$

The first term describes the interaction of the magnetic field with the electron moment and gives rise to fine structure, already discussed; while the second term concerns the electron spin-nuclear spin interaction, the origin of the hyperfine structure. More detailed examination reveals that this latter interaction may be divided into two parts, an anisotropic component and an isotropic component.

The anisotropic hyperfine splitting occurs as a result of the classical interaction of two point magnetic dipoles, and is given by:

$$\mathcal{H}^A = \frac{\vec{\mu}_e \cdot \vec{\mu}_n}{r^3} - \frac{3}{r^5}(\vec{\mu}_e \cdot \vec{r})(\vec{\mu}_n \cdot \vec{r}) \qquad (17)$$

Where $\vec{\mu}_e = -2\beta\vec{S}$, $\vec{\mu}_n = g_n\beta_n\vec{I}$, $\vec{S}$ is the spin angular momentum in units of $\hbar$ for the unpaired electron, β_n is the nuclear magneton, $\vec{I}$ is the nuclear spin angular momentum in units of $\hbar$, and $\vec{r}$ is the radius vector from the nucleus to the unpaired electron.

The isotropic interaction, first treated by Fermi,[25] comes from a nonvanishing electronic wave function density at the nucleus, and occurs only for s-type electrons. It may be written in the following manner:

$$\mathcal{H}^F = \frac{16\pi}{3} g_n \beta \beta_n |\Psi(0)|^2 \Delta(r) \vec{S} \cdot \vec{I} \qquad (18)$$

where

$$\Delta(r) \equiv \begin{cases} 1, & r = 0 \\ 0, & r \neq 0 \end{cases}$$

and $|\Psi(0)|^2$ is the probability density of the electron wave function at the position of the nucleus. By combining these terms, the Hamiltonian for the hyperfine interaction may then be written as

$$\mathcal{H}' = 2 g_n \beta \beta_n \left[\frac{3}{r^5}(\vec{r} \cdot \vec{I})(\vec{r} \cdot \vec{S}) - \frac{1}{r^3}\vec{S} \cdot \vec{I} + \frac{8\pi}{3}|\Psi(0)|^2 \Delta(r) \vec{S} \cdot \vec{I} \right] \qquad (19)$$

In the magnetic fields of a few kilogauss usually used in spin resonance work, the electronic and nuclear spins are decoupled and precess independently about the direction of the applied field. In this case, the quantum numbers

M and m may be defined as the projection of the electronic spin and the spin of the nucleus respectively, upon the field direction. Then

$$\vec{S}\cdot\vec{I} = Mm \tag{20}$$

and

$$(\vec{S}\cdot\vec{r})(\vec{I}\cdot\vec{r}) = Mm\,r^2\cos^2\theta \tag{21}$$

where θ is the angle between $\vec{r}$ and the field axis. Evaluation of the energies from equation 19 is now straightforward. Expanding to include the effects of i nuclei and using the selection rules, $\Delta M = \pm 1$, $\Delta m_i = 0$, we obtain

$$W_i' = \sum_i 2 g_{n_i} \beta \beta_{n_i} \left[\left\langle \frac{3\cos^2\theta_i - 1}{r_i^{\,3}} \right\rangle + \frac{8\pi}{3} |\psi(0)|^2 \Delta(r_i) \right] m_i \tag{22}$$

or

$$W_i' = \sum_i A_i m_i \tag{23}$$

($A_i \equiv$ coupling constant),

for the total energy of the hyperfine interaction, where $\left\langle \frac{3\cos^2\theta_i - 1}{r_i^{\,3}} \right\rangle$ is the average of the anisotropic term over the orbital of the unpaired electron. This term will, in general, be a function of the orientation of the free radical with respect to the field, while for a spherically symmetric s orbital it goes to zero.

We may now write the complete resonance condition, including hyperfine effects:

$$h\nu = g\beta H + \sum_i A_i m_i \tag{24}$$

or

$$h\nu = g\beta [H + \Delta H] \tag{25}$$

where $\Delta H = \frac{1}{g\beta} \sum_i A_i m_i$ represents the width of the hyperfine splitting in gauss, the quantity usually measured.

A primary source of the hyperfine interaction in biological free radicals is the hydrogen nucleus. For an electron in a hydrogen 1s orbital, the anisotropic contribution vanishes, leaving the isotropic term, which has a value of 507 gauss.[26] The ratio of the total isotropic splitting to 507 gauss

will then give the fraction of 1s character the unpaired electron in a free radical will have in the vicinity of the hydrogen nuclei. If the unpaired electron couples equally with n protons

$$h\nu = g\beta H + A\sum_{i=1}^{n} m_i \tag{26}$$

where $\sum_n m_i = \frac{1}{2}n, \frac{1}{2}n-1, \cdots, -\frac{1}{2}n+1, -\frac{1}{2}n.$

This will result in 2n+1 lines, with the relative intensity of each line proportional to the number of distinct arrangements of the n protons to give each value of Σm_i. The splittings between the lines measure the 1s character of the unpaired electron.

D. Configurational Interaction. In many spin resonance spectra, hyperfine splittings are observed when the application of atomic and molecular orbital theory coupled with the considerations of the previous sections would predict no, or very small, splittings would occur. Thus, an unpaired electron associated with a planar ring structure, and delocalized in the π-orbital system, above and below the plane of the ring, would be expected to have a zero wave-function density at the nuclei of the ring, and consequently no interaction with the edge protons of the ring. It is found experimentally, however, that a marked and well-resolved hyperfine structure does exist in this case.[27] The effect of configurational interaction supplies the important mechanism whereby this may occur. First, let us consider the bonding between a carbon and a hydrogen atom. Carbon, through hybridization of its atomic orbitals, may be found in any one of three equivalent bonding configurations.[28] They are designated sp, or digonal; sp^2 or trigonal; and sp^3, or tetragonal; with bond angles of 180°, 120°, and 109° 28', respectively. A bonding hydrogen atom will then form a σ bond with one of the hybrid carbon orbitals. An unpaired electron in the carbon p orbital will then have no probability

density at the proton, and hence no hyperfine splitting. However, if we allow higher order molecular orbitals of the proper symmetry to mix with the bonding σ orbital, there will also be an admixture with the orbital of the unpaired electron and hence, a finite probability density at the site of the proton, accounting for the observed hyperfine splitting. Weissman[29] and McConnell[30] have treated this configurational interaction quantitatively, with the result that the hyperfine splitting produced by a proton attached to a given carbon atom is linearly proportional to the electron density on that carbon atom. The hyperfine splitting due to interaction with this proton may then be written as

$$\Delta H = Q \rho_i \qquad (27)$$

where ρ_i is the unpaired spin density on the ith carbon atom, and Q is a semi-empirical constant, depending upon the molecule (~25 gauss for aromatic hydrocarbons).

More rigorous analysis[31] of the configurational interaction revealed the possibility of negative spin density at certain carbon atoms. This negative spin density is produced by the disturbing effect of the unpaired electron on the orbitals of the paired electrons, causing an effective unpairing of the previous balanced orbits which creates a spin density opposite in sign to the electron producing it. The algebraic sum of the spin densities remains unity, for the positive spin densities increase proportionally; however, the hyperfine splitting depends upon the sum of the magnitudes of the spin densities, which may now exceed unity, ie.

$$\sum_i \rho_i = 1 \text{, but } \sum_i |\rho_i| \geq 1 . \qquad (28)$$

E. Hyperconjugation. The previous theory is still not adequate to describe the hyperfine splitting that occurs for an electron in a carbon

ρ orbital when a methyl (CH_3) group is bonded to an sp^2 hybridized carbon atom.

Through the proper combination of the proton wave functions in the methyl group, however, a composite wave function may be formed with the same spatial symmetry as that of the carbon ρ orbital. Interaction will then occur through the overlap between the two wave functions. Since the methyl group rotates about the C-C bond, the three protons contribute equally to the splitting. This type of interaction, depending upon the overlap of wavefunctions with the same symmetry or pseudo-symmetry, is called 'hyperconjugation.'

Here again we may write for the splitting (for each proton):

$$\Delta H = Q \rho_i \tag{29}$$

where ρ_i is the spin density on the sp^2 carbon, and Q is a semi-empirical constant (~25 gauss). Thus, it may be seen that hyperconjugation is a more direct and potent effect than configurational interaction, each proton of the methyl group producing approximately the same splitting as a proton connected directly to the carbon atom in question.

F. Line Widths. Observed line widths for free radical spectra depend upon many variables: the spin-lattice interaction, dipole-dipole coupling (an anisotropic spin-spin interaction), exchange narrowing, homogeneous and inhomogeneous broadening, and anisotrophy in the g-factor.

The spin-lattice interaction involves all processes in which the electron spins may give up energy to the molecule or lattice as a whole. Most generally, the interaction takes place through the intermediary of spin-orbit coupling. The effect is usually characterized by a relaxation time, T_1, given by[32]

$$T_1 \propto \frac{\Delta^M}{\lambda^2 T^N} \tag{30}$$

where Δ is the splitting to the next orbital level, λ is the spin-orbit coupling coefficient, T the absolute temperature, M is a constant having a value of 4 or 6, and N, a constant equal to 1 or 7, depending upon whether resonance exchange or a Raman process is predominant. In free radicals, λ is quite small, and as explained in Section C, Δ is large, resulting in large values for T_1. Thus, the spin-lattice interaction is not usually strong enough to cause appreciable broadening by shortening the lifetime in the state. The reverse effect may occur, however; that is, the value for T_1 may be so large that at high power levels saturation effects occur and homogeneous broadening is produced because the spins cannot return to the lower state quickly enough. This will cause both a reduction in line intensity and a flattening of the line shape.

The anisotropic electron spin-nuclear spin interaction depends, as noted in Section D, upon the averaging of an orientation-dependent term over the orbital of the unpaired electron. For a fixed orientation of the radical within the solid, detailed analysis[33] reveals that the interaction with one proton results in a doublet splitting with two rather large subsidiary shoulders, while the interaction with more than one proton becomes rather complex, although specific cases have been calculated.[34] 'Tumbling' or rotation of the free radical in a random manner within the solid will tend to reduce the splitting. These 'tumblings' are transitions between the rotational states of the molecule induced by interaction with the surrounding lattice. Weissman[35] has shown that the anisotropic dipolar interaction can average to zero, if the 'tumbling' of the protons is greater than the frequency corresponding to the hyperfine splitting.

In the free radicals studied here, an intermediate effect was found; that is, anisotropic dipole-dipole interaction was present, but as a broadening of the lines. This was especially apparent in the effect on the observed

spectra of lowering the temperature to 77°K from 300°K. In these cases, a strong broadening of the lines was observed due to the hindered tumbling motions of the radicals at the reduced temperature.

Electron spin-spin interaction between free radicals may also occur; however, the free radical concentrations are quite small in the compounds studied, and therefore this interaction plays little role in line broadening.

A closely related effect is exchange narrowing, an interaction which results in an effective averaging of the anisotropic spin-spin interaction similar to the 'tumbling' of the radicals mentioned previously, thus reducing the line width. It also plays a significant role only for high radical concentration, the most common case being for diphenyl-picryl hydrazil (DPPH).

Inhomogeneous broadening is related to the inhomogeneity of the magnetic field at the sample. It is estimated to be within 0.1 gauss in this case and therefore will not contribute appreciably to the line widths observed.

G. Radiation Damage and the Cage Effect. Creation of the unpaired electrons observed in these studies may occur in several ways. At first, ionization of the molecule takes place either directly, through Compton and photoelectric processes, or indirectly through other secondary excitations. Once the event of radiation damage has occurred, the resulting products will then establish equilibrium with the surrounding media. This may be accomplished through reorganization of the electronic structure, or by reactions with the surrounding molecules to form more stable structures.

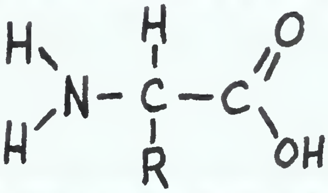
In certain cases, as in the polypeptides investigated here, the electron vacancy may migrate to the site of a proton, combine with it and escape the molecule, leaving a free radical which is stable over long periods of time. In this case the radical is trapped (the cage effect) in the material of

which it is a part. Movement of such radicals is restricted, while reaction with the cage walls only produces the products already present—hence, the most stable configuration is reached. The radicals will be free to move about within the cage, this motion being the 'tumbling' referred to in the previous section, which results in the reduction of anisotropic line broadening.

H. Polypeptide Structure.

> Amino acids in chains
> Are the cause, so the X-ray explains,
> Of the stretching of wool
> And its strength when you pull,
> And show why it shrinks when it rains.*

As early as 1800, systematic investigations of the complex substances which characterize 'living' matter were under way; and in 1844, Mulder[36] applied the term 'Protein' to these substances. The simplest amino acid, glycine, was isolated and identified as a product of protein hydrolysis in 1820, and in the succeeding years a total of 26 amino acids have been shown to result from decomposition of proteins.[37] With only slight variation, all the amino acids are characterized by the following basic structure:



where R designates a particular side chain which is the distinguishing feature of a particular amino acid. The carbon atom in the COOH group is in sp^2 hybridization and hence this group is planar, while the other carbon,

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* A. L. Patterson quoted by W. T. Astbury, Trans. Faraday Soc., 34, 378 (1938).

designated the α-carbon, is in an sp^3 or tetragonal bonding configuration. Two isomeric forms may exist, the one shown, called the L, or levo form, and its mirror image, called the D, or dextro form.

In proteins or polypeptides, the various amino acid constituents are joined together through a process in which the carboxyl (COOH) group of one amino acid loses an OH which combines with an H from the amine (NH_2) group of the next to form water and a covalent CO-NH bond. This is termed 'peptide' bonding. The phenomenon of denaturization and the stretching of fibrous proteins have pointed to the existence of other bonding factors, notably the hydrogen bond, which play a significant role in the spatial configuration for polypeptide and protein chains. The hydrogen bond involves the sharing of hydrogen atoms between the nitrogen and the carbonyl oxygen of different peptide bonds to form linkages of the type -NH. . . . . .OC-.

Bond energies for this hydrogen bond are roughly 10-20 times weaker than other bonds in the chain.

Numerous spatial configurations for the proteins and the polypeptides have been proposed, taking into account the factors mentioned plus the results of X-ray and infra-red measurements. Of these, the 'pleated sheet' structure for an extended peptide chain proposed by Pauling and Corey[38] for β-keratin corresponds closely to that assumed for the polypeptides studied in this thesis. In this model, adjacent peptide chains are joined through inter-chain hydrogen bonding to form the layer or 'sheet.'

Final conclusions as to the structure of the synthetic polypeptides studied here have not been reached; however, and therefore further discussion of this aspect will not be attempted.

The samples used in these studies were synthesized in a one-step polymerization process,[39] rather than through the laborious step-by-step classical synthesis of peptides, resulting in macromolecules with varying molecular

weights. In the discussion of results, molecular weights listed will therefore be an average for the particular sample. The structure of the individual molecules is illustrated with the associated spectra for ease in interpretation.

Chapter III

EXPERIMENTAL ARRANGEMENT

A. General. The experimental curves reported here were obtained from a standard X-band (3 cm. wavelength) spectrometer system, with a second derivative representation and rectilinear recording as provided by a Texas Instruments Corporation Recti/riter pen and ink recorder. A block diagram of the system used is shown in Figure 1.

The system is similar in principle and operation to the spectrometer described by Beringer and Castle[40] for the study of paramagnetic gasses, modified in this laboratory and adapted for use with single and polycrystalline samples. The spectrometer employs a direct transmission scheme with a barretter or bolometer detector and phase sensitive lock in detection, an arrangement which possesses the advantages of simplicity and comparatively high detection efficiency.[41]

B. The Microwave System. Microwave power is fed into the system from a Varian type VA-201B klystron operating at a frequency of 9.2 KMc. with an average power output of 50 milliwatts. External stabilization was not employed, since this klystron, when water cooled, was found to be sufficiently free from frequency drift after an approximately 30-minute warm-up period.

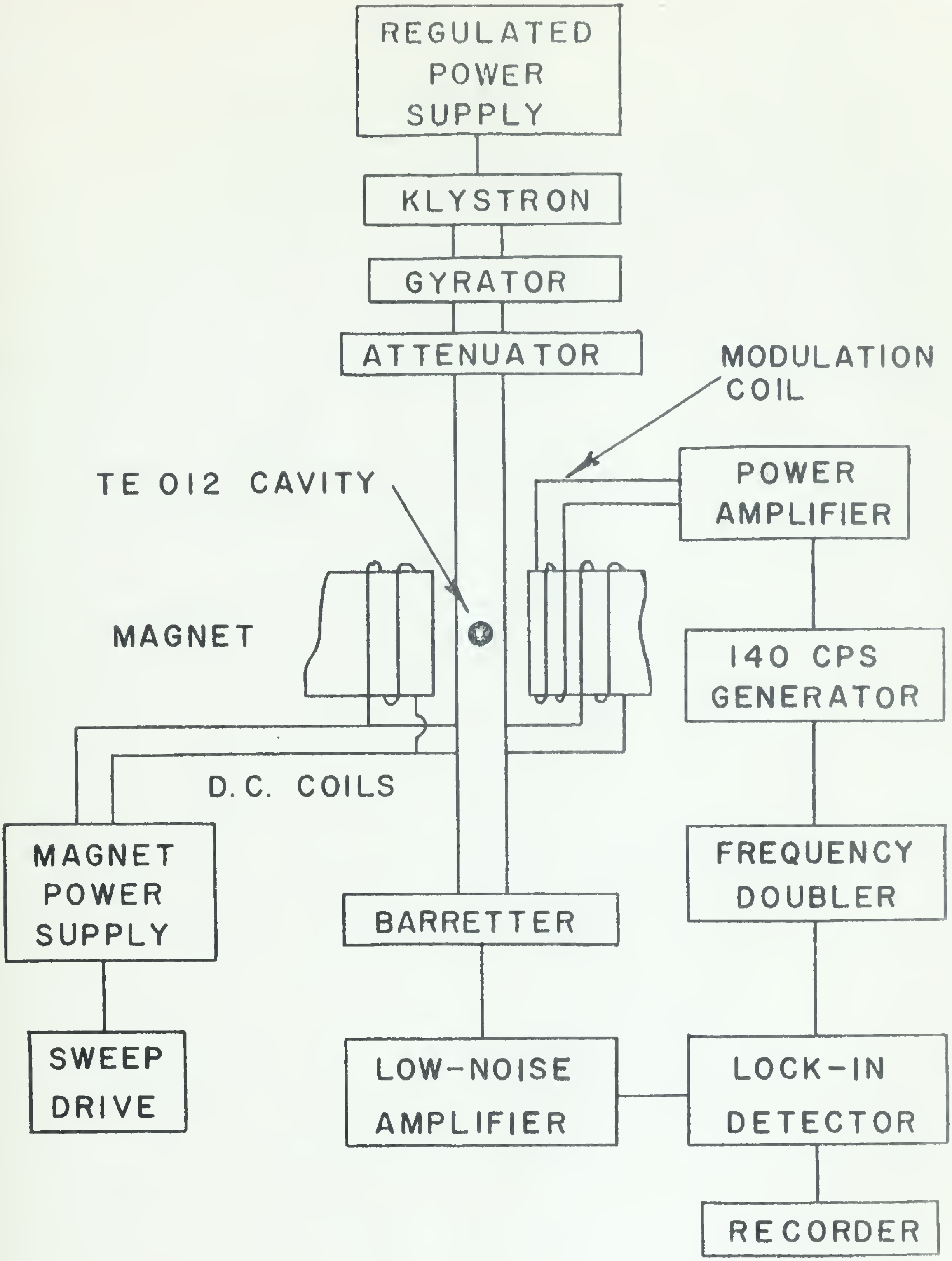


Figure 1. SPECTROMETER SYSTEM

The klystron is electronically fine tuned at the regulated power supply to match the resonant frequency of the transmission cavity with the sample in position, and is coupled to the cavity through a ferrite gyrator and an attenuator. The gyrator and the attenuator as well as those other standard microwave components which were used, whose functions and operations are well known, will not be described here.

The cavity employed is operated in the TE 012 mode, and serves to concentrate the microwave power at the sample. The cavity is shown in perspective in Figure 2, with the relative orientation of the D. C. magnetic field and the microwave magnetic field indicated at the location of the sample. It may be seen that at this position, centered in the cavity, the sample is in the maximum microwave magnetic field for maximum absorption, and also at minimum microwave electric field to reduce non-resonant dielectric losses.

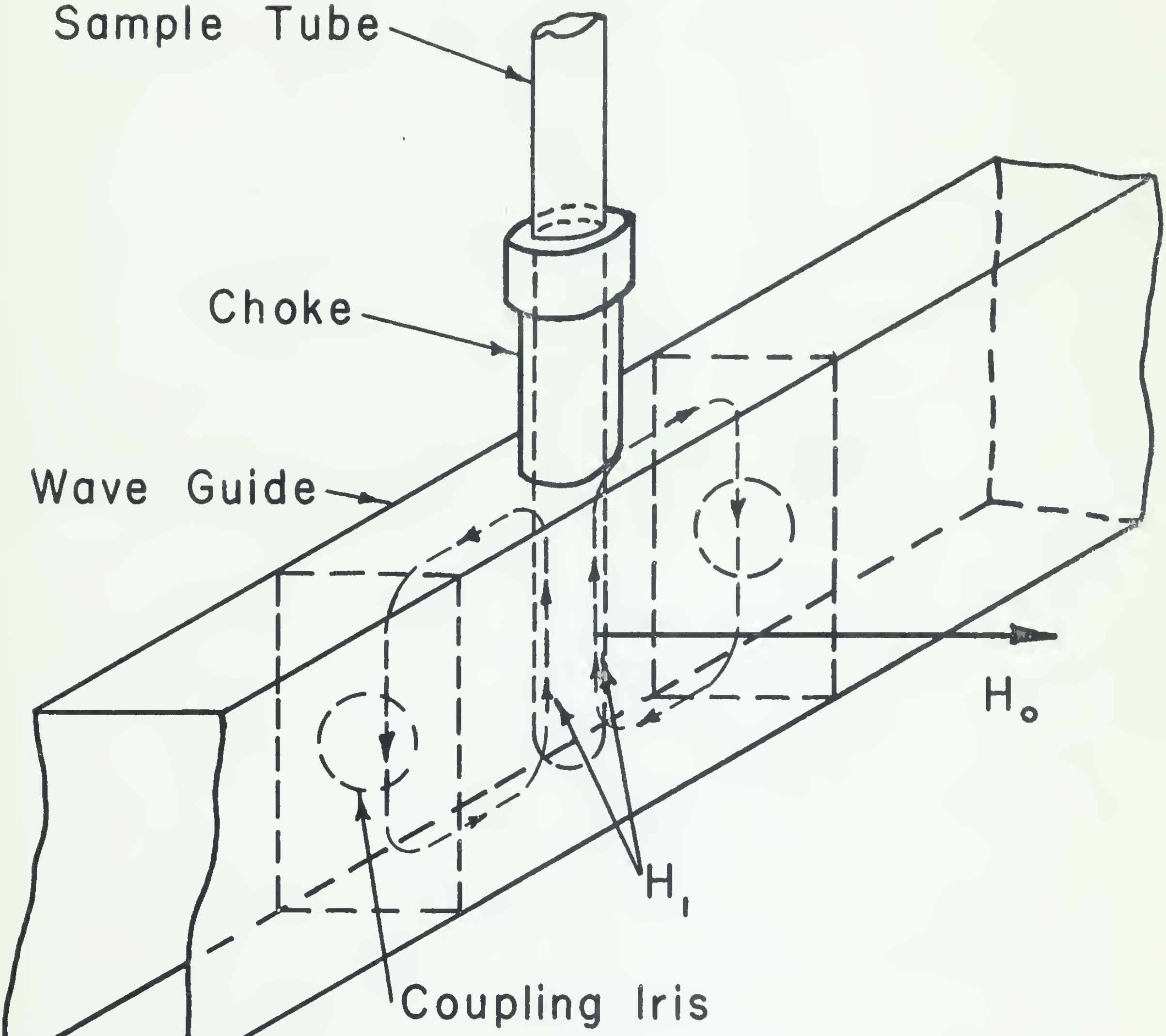
For detection of the microwave power transmitted by the cavity, a Sperry type 821 barretter is utilized. The barretter has as its active element a fine platinum wire which is mounted at, and parallel to, the maximum microwave electric field. When properly biased, the resistance of the barretter is given by

$$R = R_0 + JP^n \tag{31}$$

where $R_0 = 100$ ohms, $J = 6$ ohms per milliwatt, $n = 0.9$, and P is the total power, D. C. and microwave, dissipated in the barretter. Thus, changes in the microwave power transmitted through the cavity to the barretter are converted to voltage changes which in turn are amplified by a narrow-band low noise amplifier and fed to the signal input of a phase-sensitive lock-in detector. The barretter, being an ohmic device, displays none of the low frequency noise typical of crystal detectors, and therefore is preferable as a detector when low frequency magnetic field modulation is used, as in this

FIGURE 2. CAVITY DIAGRAM

Diagram of TE_{012} Cavity. H_1 is the microwave magnetic field, H_o, the D. C. magnetic field. Wave guide is standard X-band guide, operated in the TE_{01} mode.



CAVITY DIAGRAM

spectrometer system.

C. The Magnet System. The D. C. magnetic field necessary for observation of resonance may be found from the resonance condition, equation 9, by using a fixed frequency (ν_0) of 9.2 KMc., and a g-factor of 2:

$$H = \frac{h\nu_0}{g\beta} = 3286 \text{ gauss} \tag{32}$$

This field is supplied by an electro-magnet with 3 inch diameter pole faces and a 5/8 inch air gap, powered by a bank of storage batteries. This provides, without complex regulating circuits, a relatively stable field which is homogeneous over the volume of the sample to within 0.1 gauss, a figure which is quite satisfactory for the polycrystalline samples used, since line widths greater than 2 gauss may be expected.

To obtain the complete resonance pattern for g values differing slightly from 2, with a fixed microwave frequency, the resonance condition indicates that the D. C. magnetic field must be varied. To accomplish this, the current to the field coils of the magnet is taken from a bank of 6AS7 tubes whose grid bias is controlled by a sweep circuit driven in turn by a constant speed clock motor. This permits a linear sweep of the magnetic field over a range of 200-300 gauss at a rate of approximately 30 gauss per minute. Further stabilization of the field is accomplished in the sweep circuit through the use of a degenerative feedback loop.[42]

For detection purposes, low frequency variable amplitude magnetic field modulation is employed. An audio oscillator generates a 140cps. sine-wave which is amplified by a power amplifier and applied to a small modulation coil on the magnet. When passing through an absorption line, this magnetic field modulation is converted to an amplitude modulation of the transmitted microwave power in the usual manner. Because of the non-linear character of

the absorption curve, however, the amplitude modulated microwave power transmitted will now not only possess variations at the fundamental modulation frequency, but will include higher harmonics of the modulation frequency as well.

Proper selection of the fundamental or the first harmonic frequency in the lock-in detector will then give signals proportional to the first or second derivative of the absorption line shape. This may be seen from a Fourier analysis of the amplitude of the transmitted microwave power in terms of the modulation frequency. Thus, if the magnetic field varies with small amplitude as A cos ωt about a point H_o, the intensity of the microwave power P(H), will be given by (using a Taylor expansion):

$$P(H) = P(H_o) + P'(H_o) A \cos \omega t + \frac{P''(H_o)}{2} A^2 \cos^2 \omega t + \cdots \quad (33)$$

which may be written in the form

$$\begin{aligned} P(H) = & \left[P(H_o) + \frac{A^2}{4} P''(H_o) + \cdots\right] \\ & + \left[A P'(H_o) + \frac{A^3}{8} P'''(H_o) + \cdots\right] \cos \omega t \quad (34) \\ & + \left[\frac{A^2}{4} P''(H_o) + \frac{A^4}{48} P^{IV}(H_o) + \cdots\right] \cos 2\omega t + \cdots \end{aligned}$$

The second term in brackets indicates the variation at the fundamental frequency is primarily due to the first derivative of the line shape, while the third term in brackets shows that the second derivative of the line shape will be the primary factor in the amplitude of the first harmonic of the modulation frequency. Thus, selection of the first harmonic should then give a signal which varies as the second derivative of the line shape.

This second derivative representation was chosen for several reasons:

(1) The peak of the second derivative and the peak of the line shape occur at the same value of the magnetic field;

(2) Weak hyperfine structure is more easily detected than in first

derivative recordings;

(3) The appearance of the spectrum obtained more closely resembles the actual absorption curve.

D. The Detection and Recording System. Final detection of the resonance absorption is accomplished by a phase-sensitive lock-in detector. Two signals are fed into the lock-in detector, a reference signal of 280 cps. originating at the audio oscillator via a standard frequency doubler, and the amplified output of the barretter. These signals are both amplified internally, the input signal from the barretter by a narrow band amplifier tuned to 280 cps. by the use of a twin-tee rejection filter in a negative feedback loop; and the reference signal in a circuit which provides for a variable phase shift of 0 to 180 degrees. These two signals, properly phased, are then applied to a Schuster type[43] phase-sensitive mixing section, where a form of gating action takes place, in which the signal amplitude is only allowed through the stage by the peaks of the reference signal, thus rejecting random noise beats which are not phase-coherent with the reference signal. The output of this stage is then applied to one of a group of RC networks to permit selection of an appropriate time constant and then to a standard push-pull D. C. amplifier which in turn provides the signal proportional to the second derivative of the line shape for the recorder.

With the detection system just described, the sensitivity of this spectrometer is such that $\sim 10^{15}$ unpaired spins may be detected.

E. Experimental Techniques. Since the presence of air was found to have a strong quenching effect upon the free radicals formed in the polypeptides used, all samples were initially irradiated and observed in a vacuum, and then the effect of opening to air obtained.

The position for the g-value marker (indicated by an arrow at the top

of each spectrum illustrated) was located by superimposing the single sharp, strong line of a small polycrystalline sample of diphenyl-picryl-hydrazil (DPPH) on the spectrum of the sample. The g-value for polycrystalline DPPH has been accurately measured,[44] and is listed as

$$g = 2.0036 \pm 0.0002 . \tag{35}$$

Line spacings were determined by calibration of the magnetic field after each run by using a sample of zinc sulfide, with Mn^{2+} as an impurity. The Mn^{2+} ion gives a spectrum of six lines roughly centered about $g = 2$ whose spacings have been accurately determined.[45] The g-value for any line may then be determined quite simply by comparison with the DPPH marker, by making use of the field calibration to determine ΔH, the splitting in gauss from the marker, and the relation

$$g\,(\text{unknown}) = 2.0036 \left(1 + \frac{\Delta H}{3286}\right) . \tag{36}$$

Measurements at 77°K were made with the aid of a special Dewar flask, constructed with a projection fitting the opening in the microwave cavity into which small sample tubes may be placed while still remaining immersed in liquid nitrogen. Boiling of the liquid nitrogen causes extra noise from vibration of the sample tube in the cavity and was checked by pumping on the flask and super-cooling the liquid nitrogen just prior to each run.

Both room temperature and liquid nitrogen sample tubes, as well as the Dewar flask projection were constructed from Corning type 7070 glass which has an especially low attenuation of microwave energy.

The free radicals observed were produced by irradiation in a kilocurie Cobalt 60 source, with dosages ranging from 2-5 million roentgens. Primary radiation from this source consists of a 1.17 Mev. γ-ray and a 1.33 Mev. γ-ray.

Chapter IV

RESULTS

A. General Discussion. A number of synthetic polypeptides have been irradiated both at 77°K and 300°K and their spin resonance patterns have been observed. Early efforts to observe spin resonance effects for these samples at room temperatures and open to the atmosphere resulted in failure; therefore, all samples were initially observed in a vacuum and then the effect of opening to air observed for each one. Further discussion of the important effects of air or oxygen will be given in Section I.

Since the primary radicals (see Chapter I) produced by irradiation at 77°K are generally different from those observed at 300°K, each substance was observed as it warmed from 77°K to room temperature in order to observe the effect of reducing the hindered rotation (causing dipole-dipole broadening), and to observe radical changes as the spin density migrates within the molecule. After the radical has stabilized at 300°K, the temperature is reduced to 77°K again to check whether a change in the radical has taken place, or whether the increased molecular motions at 300°K have just changed the appearance of the spectrum while the basic form of the radical remains unaltered. An irreversible change in the spectrum would indicate that the

free radical or radicals produced at 77°K have changed in warming to ones with a chemically different form.

Measurements on single crystals of organic compounds[46, 47] have shown the complex spectra which may result for varying orientations of the radical with respect to the field, and yet give a deceptively simple appearing spectrum in powder form. For this reason, identification of the radical or radicals contributing to a powder spectrum must be done with caution. Nevertheless, in these studies certain radicals were established with a high degree of certainty, and others will be postulated, whenever possible, to account for the spectra observed; however, as mentioned in Chapter I, the important value of a study of this nature arises in the establishment of the characteristic behavior of individual amino acids when linked in long, protein-like, peptide-bonded molecules.

Two results of these studies deserve particular mention. First, with the exception of poly-L-proline and the ringed compounds poly-L-tyrosine and poly-L-tryptophan, the spectra observed for the amino acids in these long chain molecules was found to differ considerably from those observed for the amino acids themselves. If, then, a particular amino acid residue is contributing to the spin resonance pattern of a protein or other similar peptide-bonded molecule, it would be expected to exhibit its characteristic peptide spectrum (as illustrated in the following sections), rather than the spectrum of the simple amino acid. Of the polypeptides investigated here, however, only poly-glycine and poly-sarcosine gave patterns identical to one of the patterns found for proteins. This provides further verification of the hypothesis that, in proteins at 300°K, the electron vacancies caused by the radiation migrate within the molecule to the glycine (or sarcosine) residues where one of the protons is lost and the characteristic glycine-type

radical is formed. A similar effect involving residues containing sulphur could not be verified because of the unavailability of the associated polypeptides.

The second result concerns the quenching effect observed for all the polypeptide resonances when evacuated samples are exposed to air or oxygen. Again, this behavior is not characteristic of the simple amino acids, but may be found, to a somewhat lesser extent, in the proteins, the nucleic acids, and other organic material. Examination of the mechanism of the oxygen effect (Section I) reveals how spatial configuration plays an important role in determining the degree to which the spectrum is modified by oxygen attack.

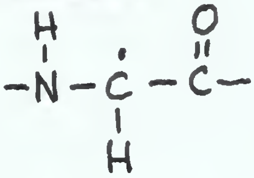
Particular aspects of the spectra observed for individual polypeptides will be presented in the following sections. In these sections, compounds with similar molecular structure or chemical behavior have been grouped together.

B. The Glycine Group: Poly-Glycine and Poly-Sarcosine. Poly-glycine is the simplest of the polypeptides. The basic unit, glycine, is widely distributed among the proteins and appears in especially large proportions in collagen and elastin. The samples used here had an average molecular weight of 3,000 and hence the peptide chains contained an average of 52 glycine residues. Since the end groups of the simple amino acids play an important role in the radicals produced, it is apparent that in this case end group effects are negligible.

Included in this section with poly-glycine is poly-sarcosine (N-methylglycine), a substance of similar structure found in peanut proteins and in an antibacterial agent, actinomycin. The average molecular weight for polysarcosine is 2,500, so the peptide chains are ∼25 units in length, and

again end group effects may be neglected. The spectra of poly-glycine at 300°K and at 77°K are shown in Figure 3; of poly-sarcosine in Figure 4. A segment of the molecule is indicated in each figure for ease in identification of the radical; no effort has been made to indicate a spatial configuration, bond angles, bond lengths, or hydrogen bonding where it is expected to exist.

Both poly-glycine and poly-sarcosine display a doublet spectra at room temperature with an identical splitting of 17 gauss between components. This suggests a radical of the form



$$-\overset{\displaystyle H}{\overset{|}{N}}-\underset{\underset{\displaystyle H}{|}}{\dot{C}}-\overset{\displaystyle O}{\overset{\|}{C}}-$$

for poly-glycine where coupling with the single proton on the α-carbon results in the doublet observed. Early observations on di-and tri-peptides of glycine, and the later analysis of the doublet pattern in oriented silk strands tend to confirm this result. In the report on silk, Shields and Gordy[48] have calculated the isotropic Fermi coupling constant to be $A_f = 17$ gauss, assuming the same radical is produced, and then proceed to account for the orientation dependence of the doublet splitting on the basis of anisotropy in the coupling constant. This work indicates that 17 gauss is indeed the isotropic splitting to be expected for poly-glycine in peptide chains, while the observed line widths of ~ 8 gauss may be attributed to the anisotropic effects which broaden the lines in this powder sample. In a vacuum, this radical in poly-glycine is quite stable, with no detectable decrease in line intensity over a period of days. When the sample is opened to air, however, the intensity of the resonance begins an immediate decay (see Figure 5) which continues until the signal is no longer detectable. No evidence of

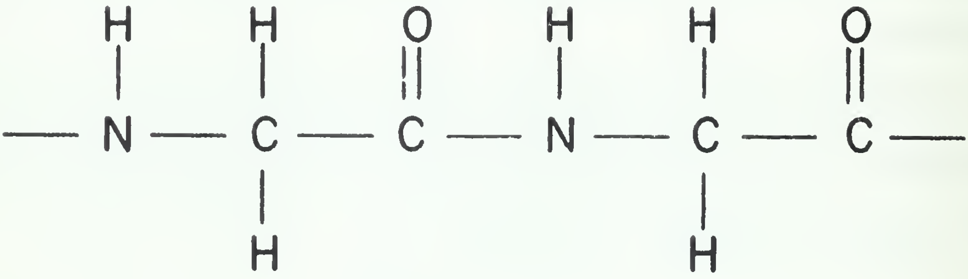
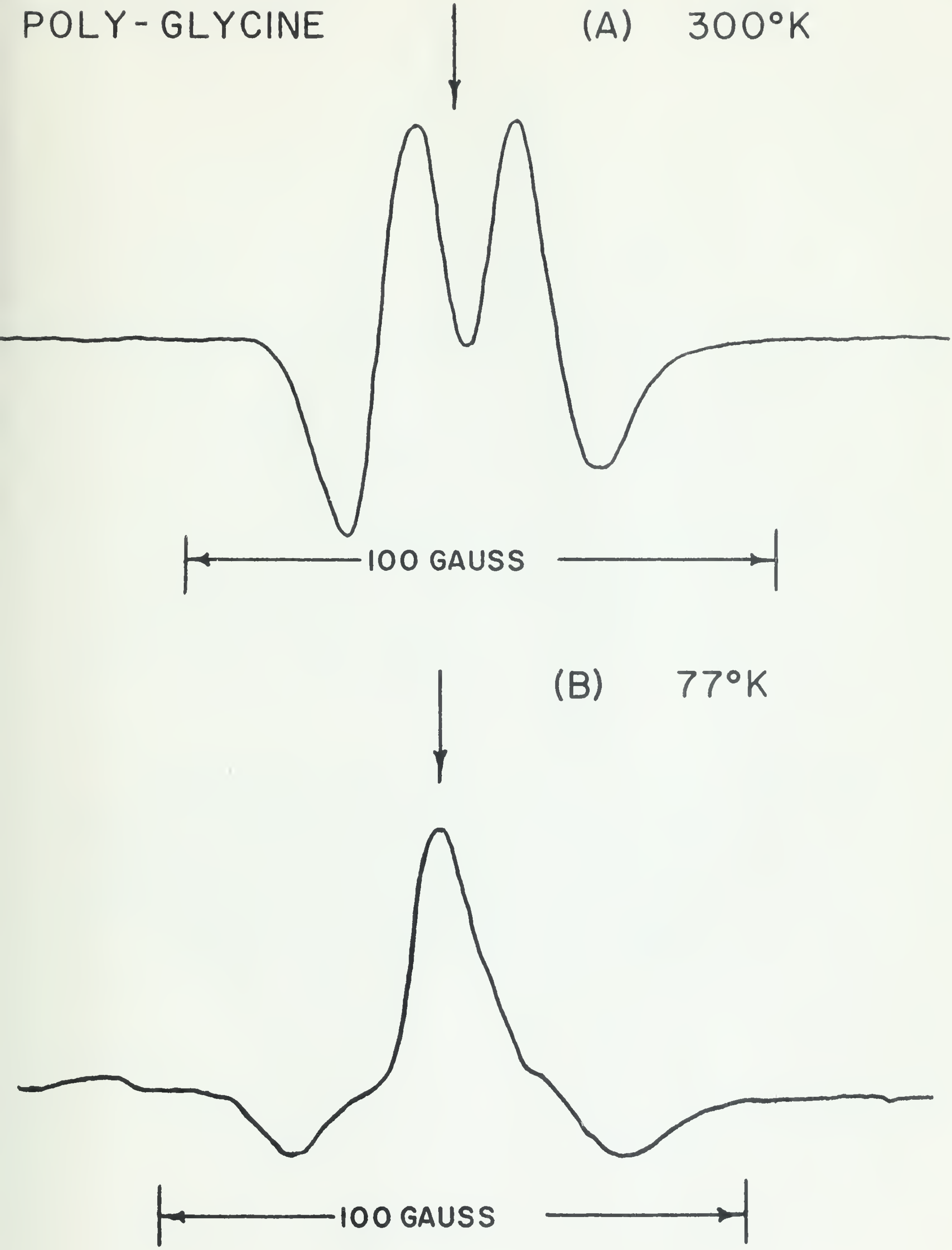


Fig. 3 (Above) Segment of a Poly-Glycine molecule.
(Facing Page) Electron spin resonance curves for an evacuated sample of Poly-Glycine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)


POLY-GLYCINE
(A) 300°K
100 GAUSS
(B) 77°K
100 GAUSS

[illegible]

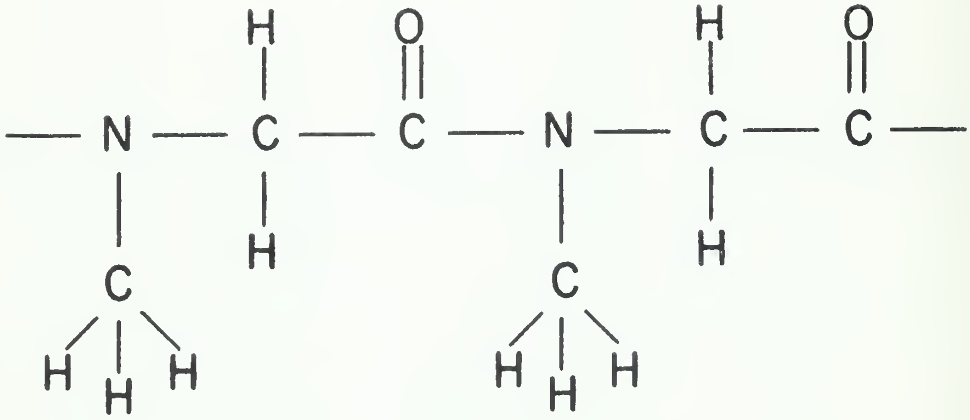


Fig. 4 (Above) Segment of a Poly-Sarcosine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-Sarcosine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

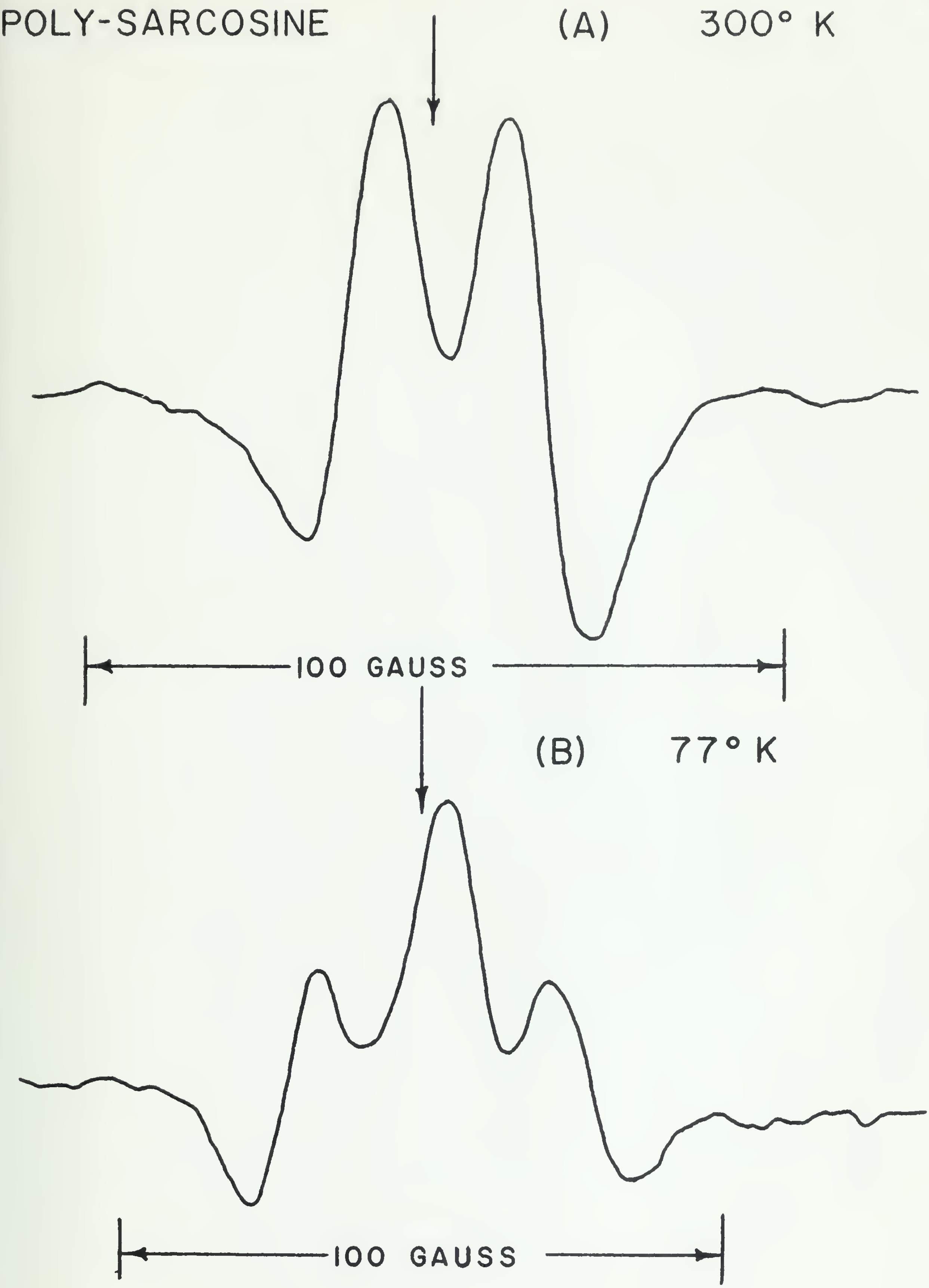
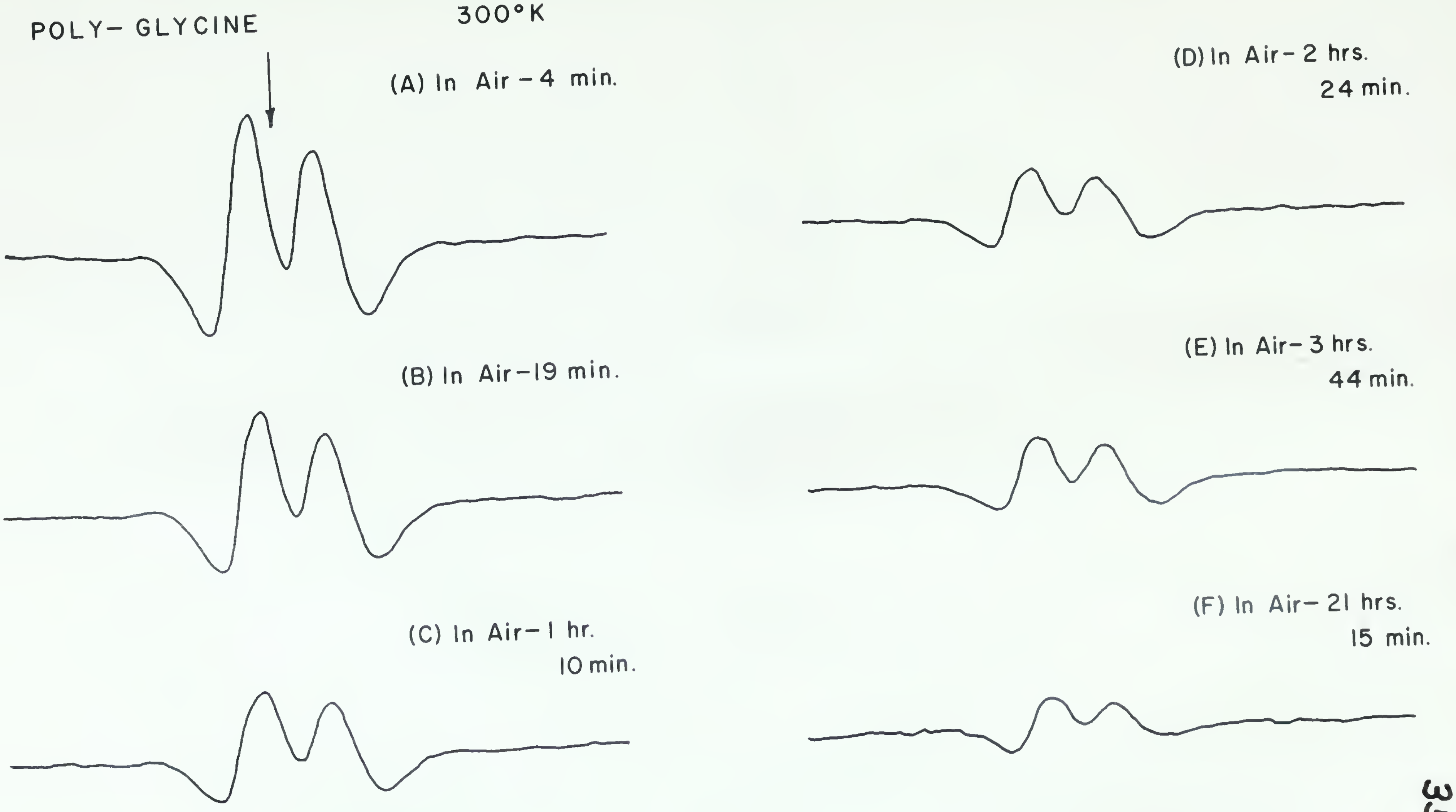
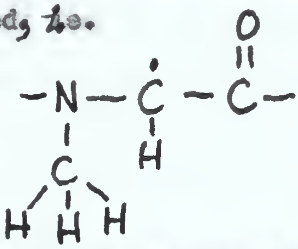

POLY-SARCOSINE
(A) 300° K
100 GAUSS
(B) 77° K
100 GAUSS

FIGURE 5. OPEN TO AIR SEQUENCE: POLY-GLYCINE
(A)-(E) at same spectrometer gain, sample unmoved.
(F) Next day, roughly the same gain settings. Splitting between components remains at 17 gauss throughout the sequence.


POLY- GLYCINE
300°K
(A) In Air - 4 min.
(B) In Air-19 min.
(C) In Air-1 hr.
10 min.
(D) In Air- 2 hrs.
24 min.
(E) In Air- 3 hrs.
44 min.
(F) In Air- 21 hrs.
15 min.

the formation of a slightly asymmetrical singlet characteristic of oxygen bonding (see Section I) is found for poly-glycine; however, the observed decay of the radical when opened to air indicates a positive effect. Since poly-glycine is known[49] to have a tightly packed structure, diffusion of molecular oxygen to the sites of radiation damage in the molecule may very well be inhibited to such an extent that recombination processes occur at nearly the same rate, resulting in a reduction in signal intensity without the concentration of bonded oxygen molecules becoming large enough to give rise to a detectable singlet. Thus, this would appear to be a verification that spatial configuration within the molecule plays an important role in the effect of oxygen on biological substances.

Analysis of the possible radicals that may be produced in poly-sarcosine and still yield a glycine-like doublet leads to the conclusion that a similar radical must be formed, i.e.



Comparison with poly-glycine, the identical component splitting of 17 gauss for poly-sarcosine, and the corresponding behavior when the sample is opened to air, help to verify the form of the radical in both compounds.

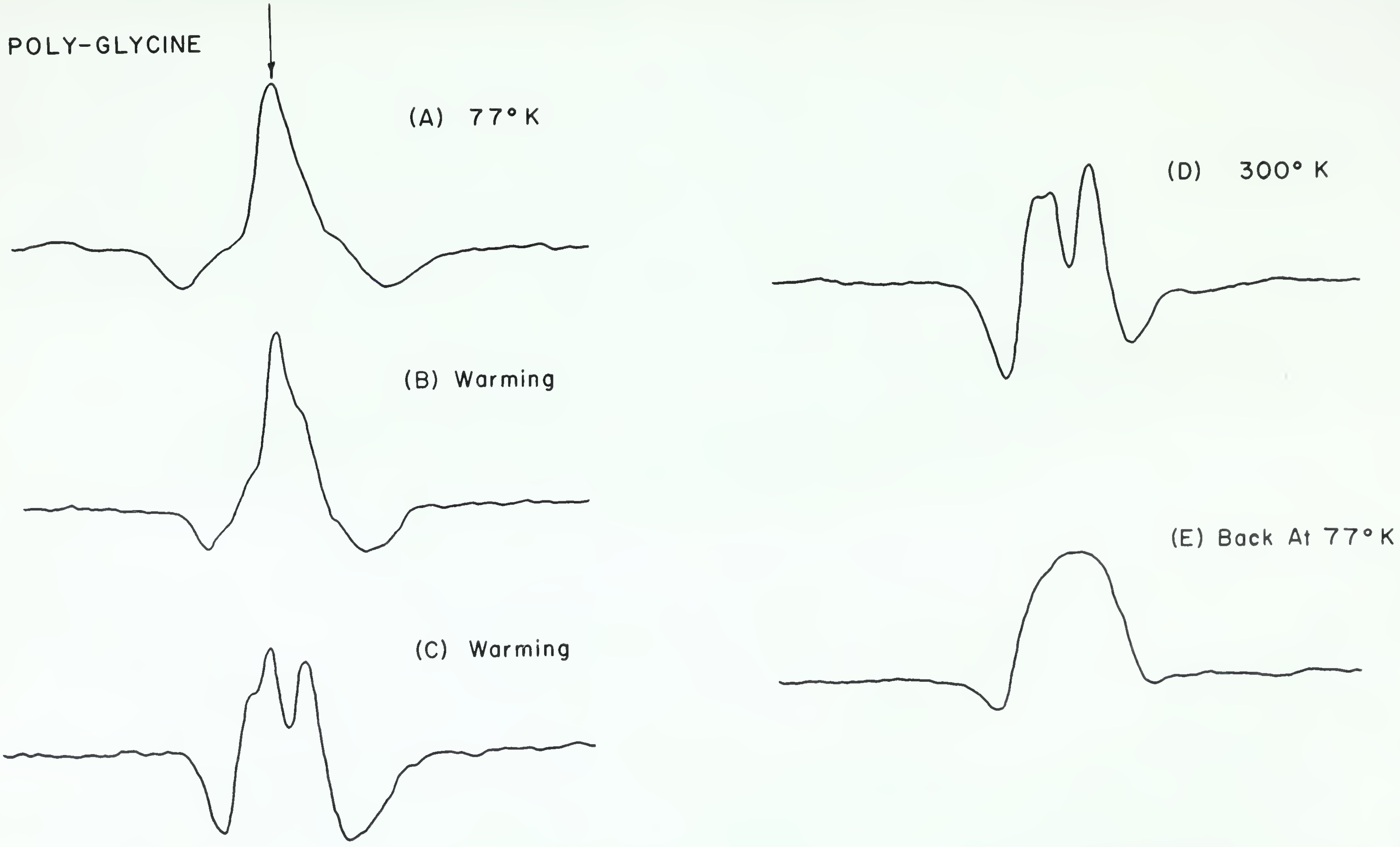
When the sample is irradiated and observed at 77°K, the spectrum of poly-glycine now displays a prominent singlet with weak, broad, and unresolved shoulders centered about $g \simeq 2.0036$. The exact nature of the radical or radicals produced in this case is unknown. The radiation producing the damage is sufficiently energetic to break any of the bonds or ionize the molecule, and a combination of these primary effects is likely to be contributing to the observed pattern. At this temperature, the most stable configuration for

the unpaired spin produced by the radiation is not formed because migration of the electron vacancy is hindered. When the sample is warmed to 300°K (see Figure 6), initially the central single line sharpens due to the decrease in anisotropic dipole-dipole coupling caused by the increased molecular motions, and then suddenly the doublet characteristic of glycine appears. This would indicate that at this point, a significant mobility of the unpaired spins is allowed to permit migration and breaking of the hydrogen bond on the α-carbon. When completely warmed, the sample shows the typical poly-glycine doublet with the exception of an apparent doubling of the low-field component. At 77°K again, this pattern is replaced by a broad smeared-out resonance, showing a strong anisotropic dipole-dipole effect.

For poly-sarcosine, the observed spectrum at 77°K is a broad triplet with intensities roughly 1:2:1. Here again this may result from the contributions of several effects. When the sample is warmed, the spectrum changes to the original room temperature pattern, exactly. Reducing the temperature to 77°K again causes no appreciable change in the spectrum. No satisfactory explanation can be forwarded at this time for this apparently contradictory behavior for these similar compounds in the process of warming and then cooling again to 77°K.

C. The Alanine Group: Poly-L-Alanine and Poly-DL-Alanine. Poly-L-Alanine and the polypeptide formed from the mixed isomers, poly-DL-alanine, gave nearly identical spectra, as shown in Figures 7 and 8. Average molecular weights of the two compounds were 5,000, indicating molecules of approximately 70 units in length, so that effects from end groups were negligible. The basic unit, alanine, is one of the more widely distributed of the amino acids among the proteins, being found in collagen, hemoglobin, insulin, egg albumin, and many others. It forms approximately 30% by weight

FIGURE 6. WARMING SEQUENCE: POLY-GLYCINE
(D) After two days at 300°K. (E) Returned to 77°K for 6 hours. Spectrometer gain remains the same, (A)-(E).


POLY-GLYCINE
(A) 77° K
(B) Warming
(C) Warming
(D) 300° K
(E) Back At 77° K

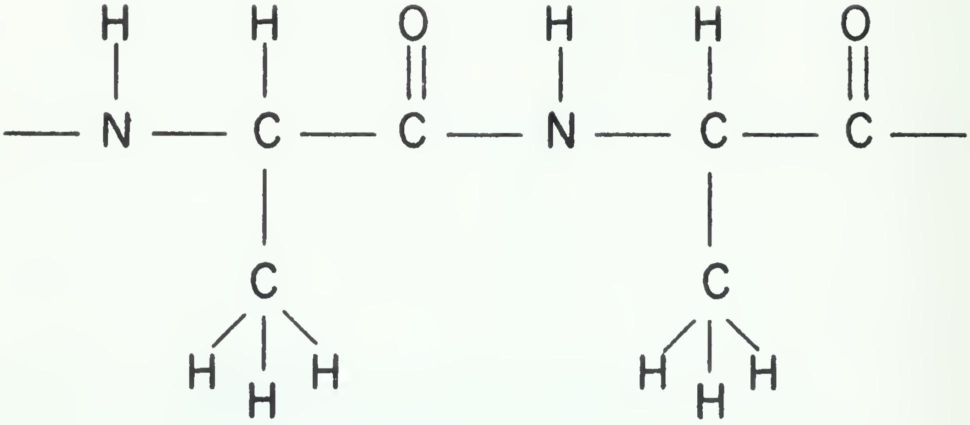
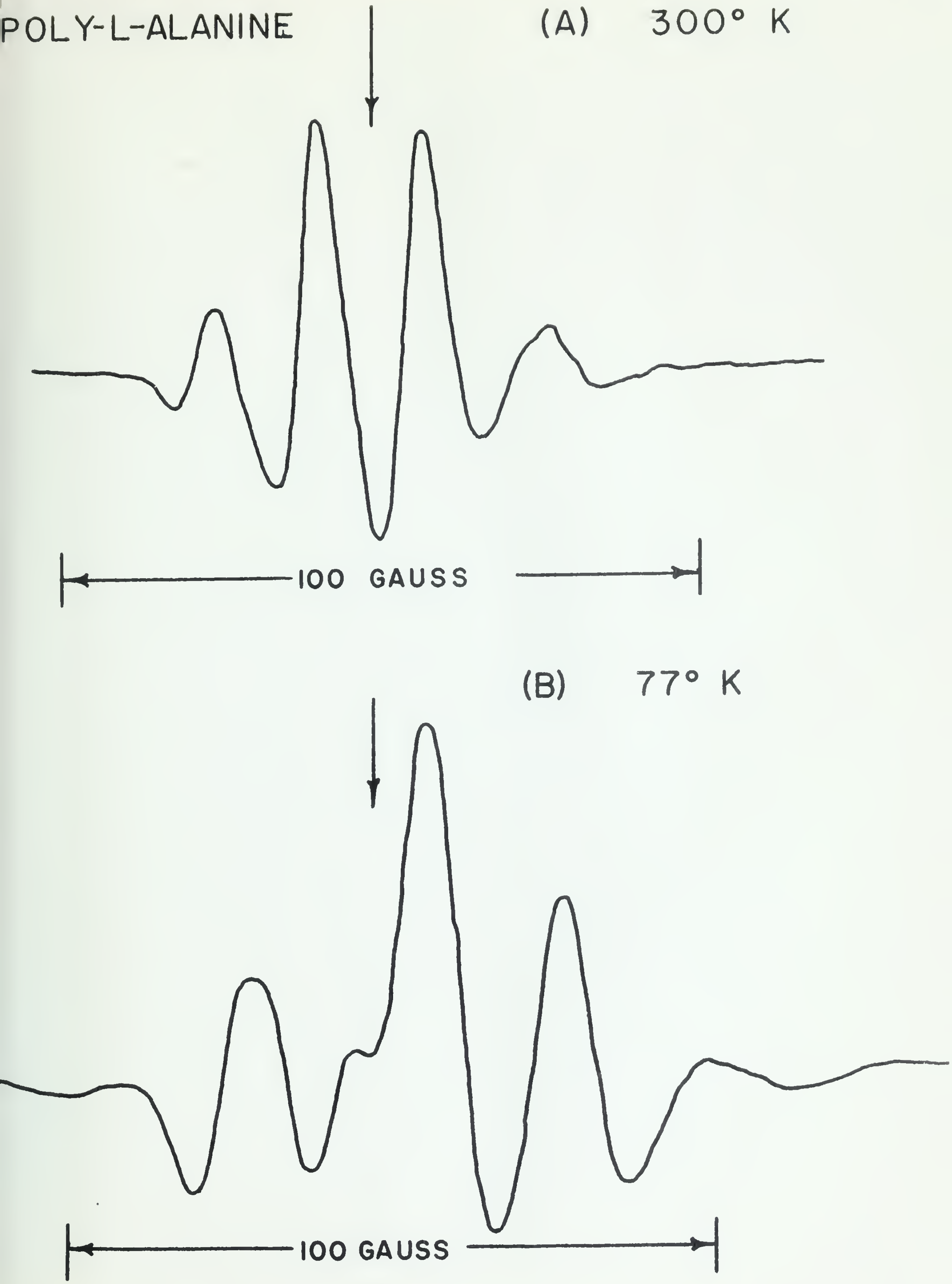
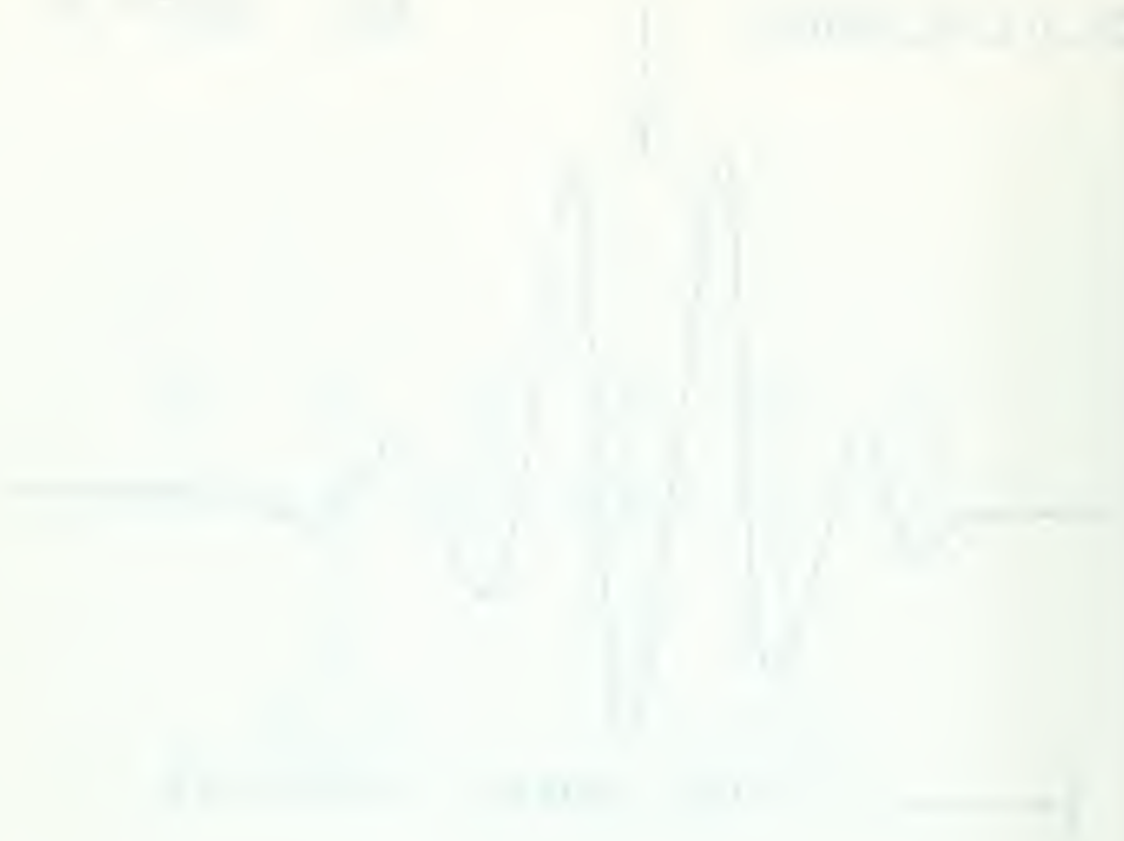
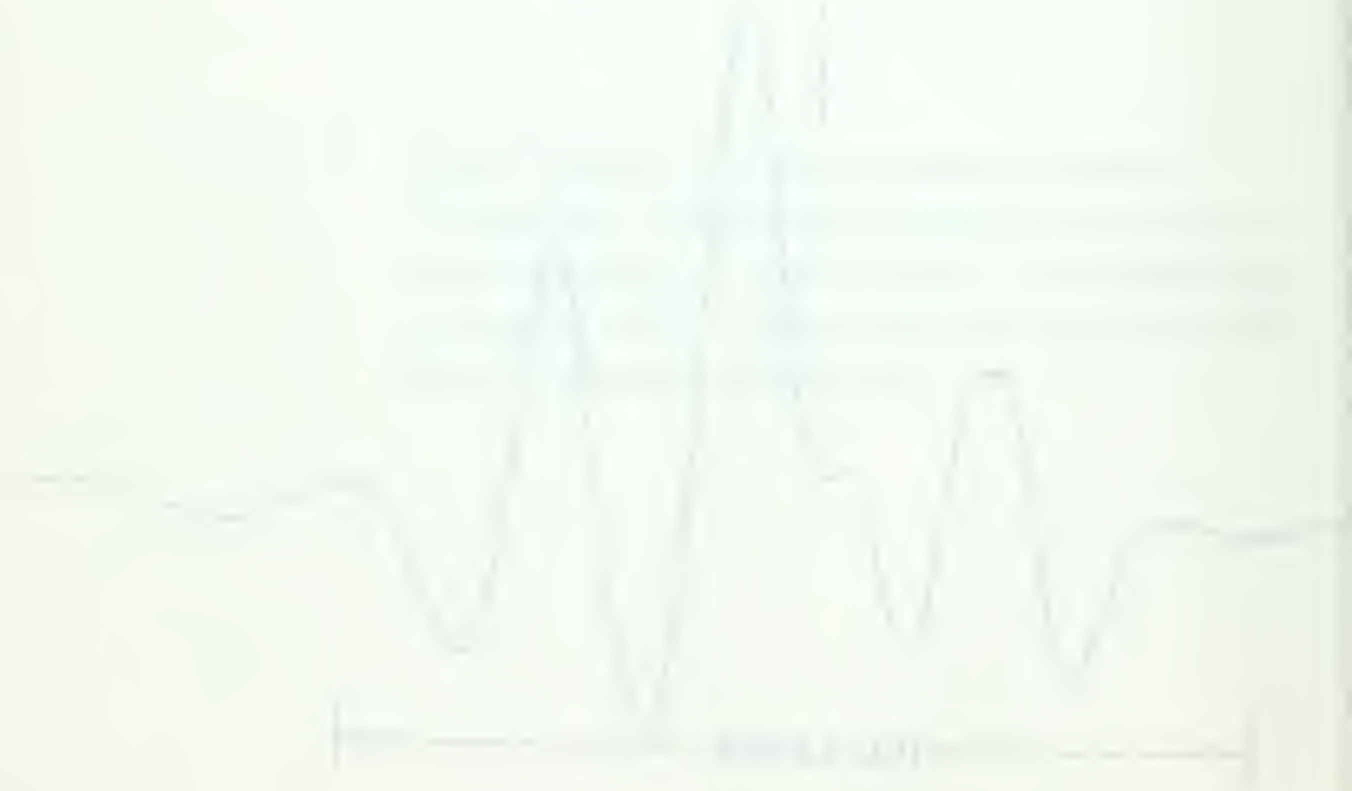


Fig. 7 (Above) Segment of a Poly-L-Alanine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Alanine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)


POLY-L-ALANINE
(A) 300° K
100 GAUSS
(B) 77° K
100 GAUSS





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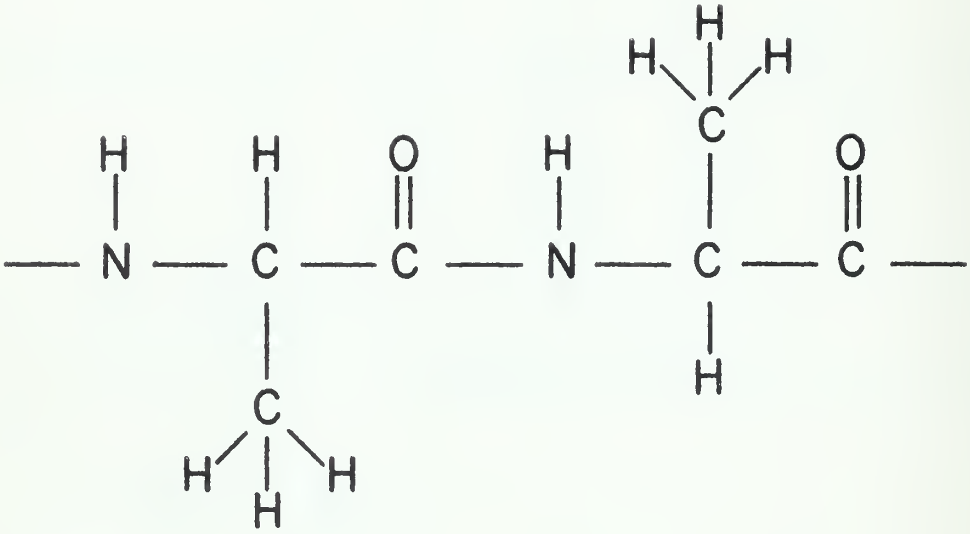
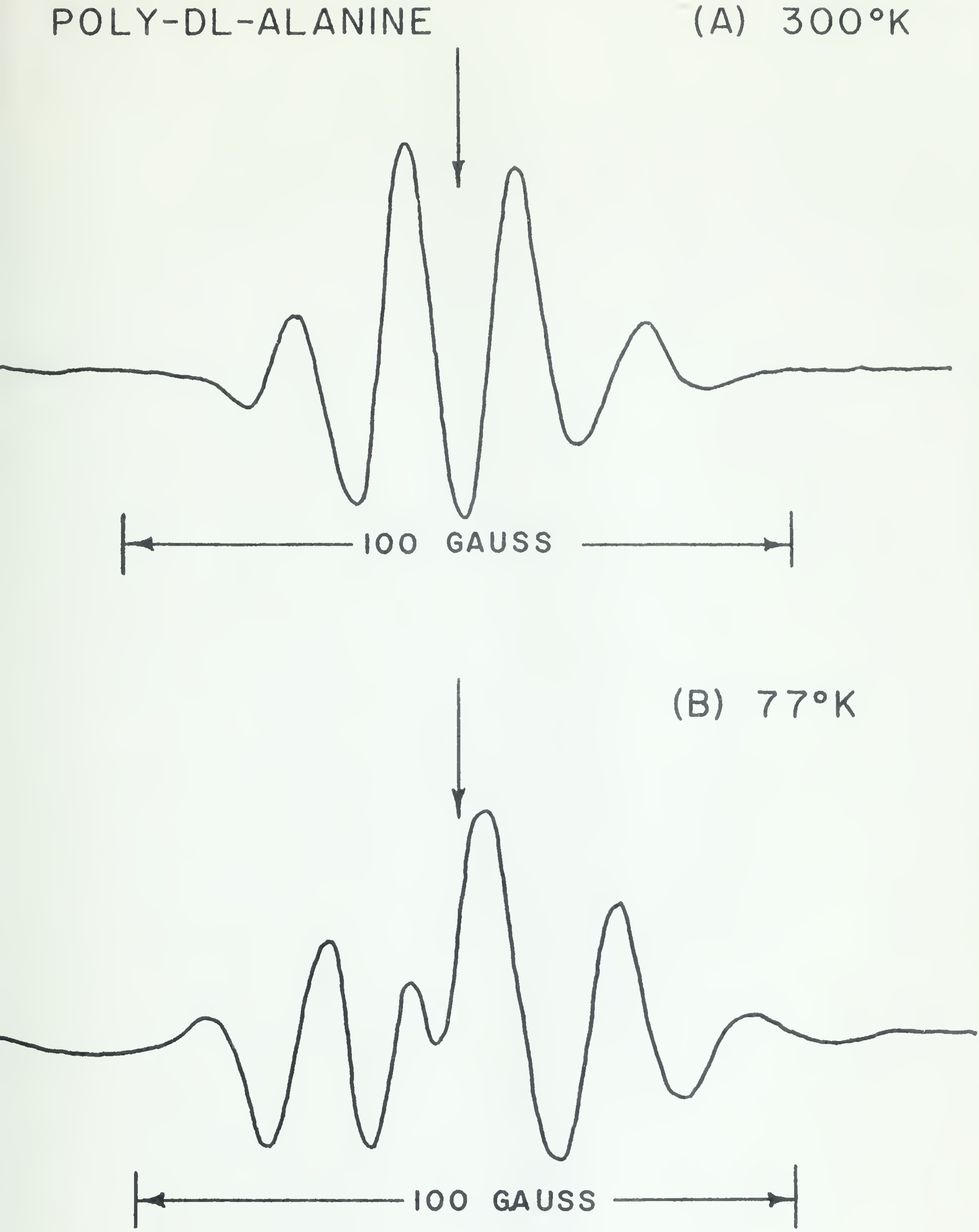
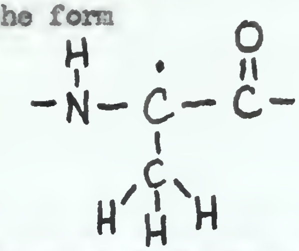


Fig. 8 (Above) Segment of a Poly-DL-Alanine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-DL-Alanine: (A) Irradiated and observed at 300^{0}K. (B) Irradiated and observed at 77^{0}K. (Second derivative representation.)


POLY-DL-ALANINE
(A) 300°K
100 GAUSS
(B) 77°K
100 GAUSS

of the composition of silk fibroin.

At 300°K, the spectrum is a quartet with line intensities approximately 1:3.5:3.5:1 and a splitting of 18.0 gauss between components. This suggests immediately a radical of the form



with equal coupling of the unpaired electron with the three protons of the methyl group through the mechanism of hyperconjugation. The methyl group is thought to be rotating about the C-C bond sufficiently at this temperature to average out any anisotropic coupling, so that just the isotropic Fermi coupling remains. Using the relation for the spin density, equation 29, and a proportionality constant, Q, of 26 gauss,[50] we find

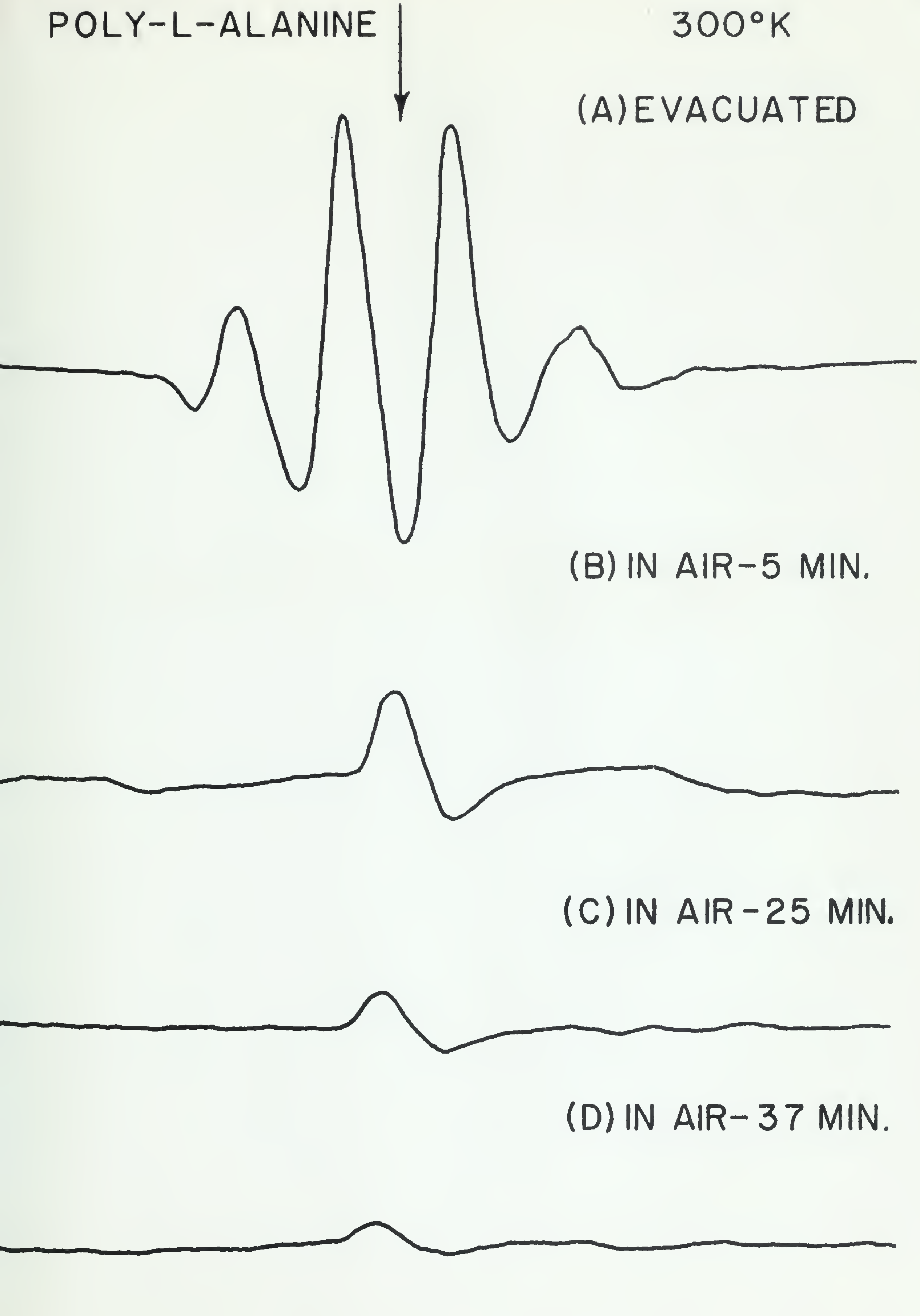
$$\rho_i = \frac{\Delta H}{Q} = \frac{18}{26} \qquad (37)$$

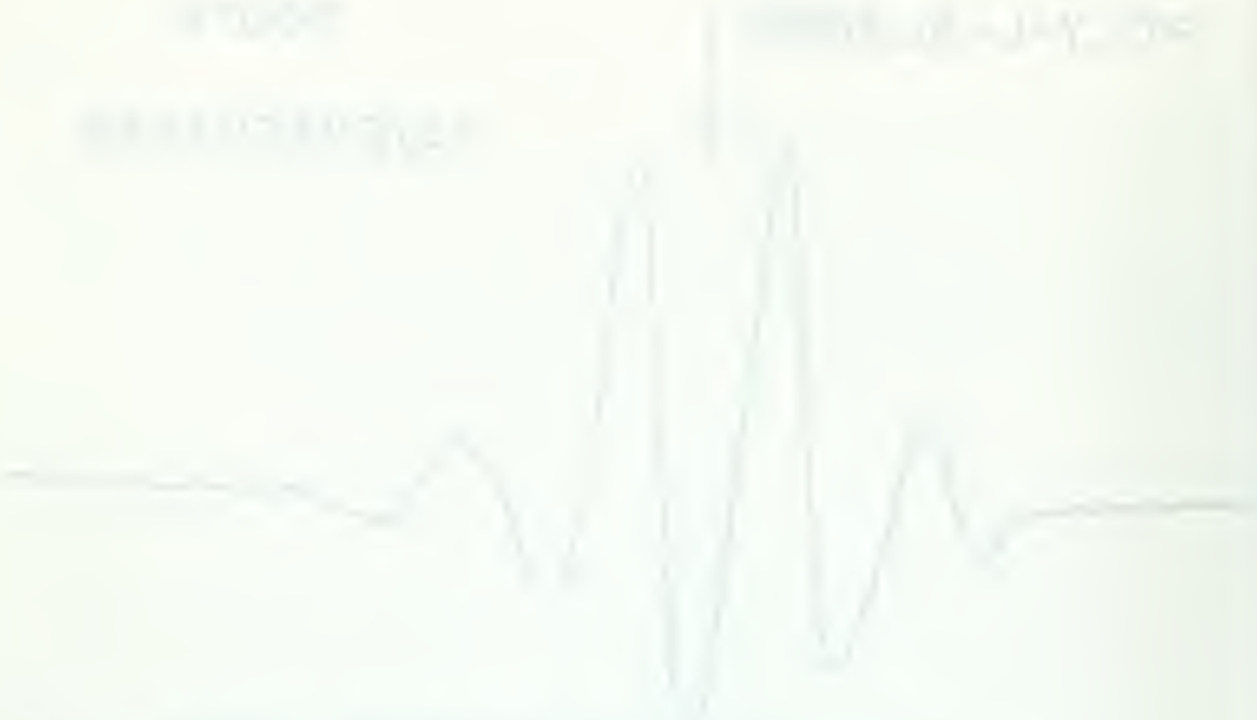
or a spin density of 69% on the α-carbon. The variation in line intensities from the ideal 1:3:3:1 pattern expected for the methyl radical would indicate a slight mixing of a radical in which the methyl group is removed and the unpaired electron interacts with the remaining proton.

Contrary to the behavior of single crystals of alanine or alanine itself, the observed room temperature spectrum is affected quite strongly by the admission of air to the sample. Within 5 minutes the strong quartet is replaced by a rather weak singlet which then continues to decay and disappears(see Figure 9). Further explanation of this 'oxygen effect' and its importance will be found in Section I.

When the sample is irradiated and observed in a vacuum at 77°K, the basic character of the spectra is altered. The primary form is now that of a quintet, with the addition of a small line on the shoulder of the central peak , split

FIGURE 9. OPEN TO AIR SEQUENCE: POLY-L-ALANINE
Spectrometer gain remains the same in (A)-(D). Behavior of poly-DL-alanine is similar. Times listed are to the center of the resonance.


POLY-L-ALANINE
300°K
(A) EVACUATED
(B) IN AIR-5 MIN.
(C) IN AIR-25 MIN.
(D) IN AIR-37 MIN.



[illegible]

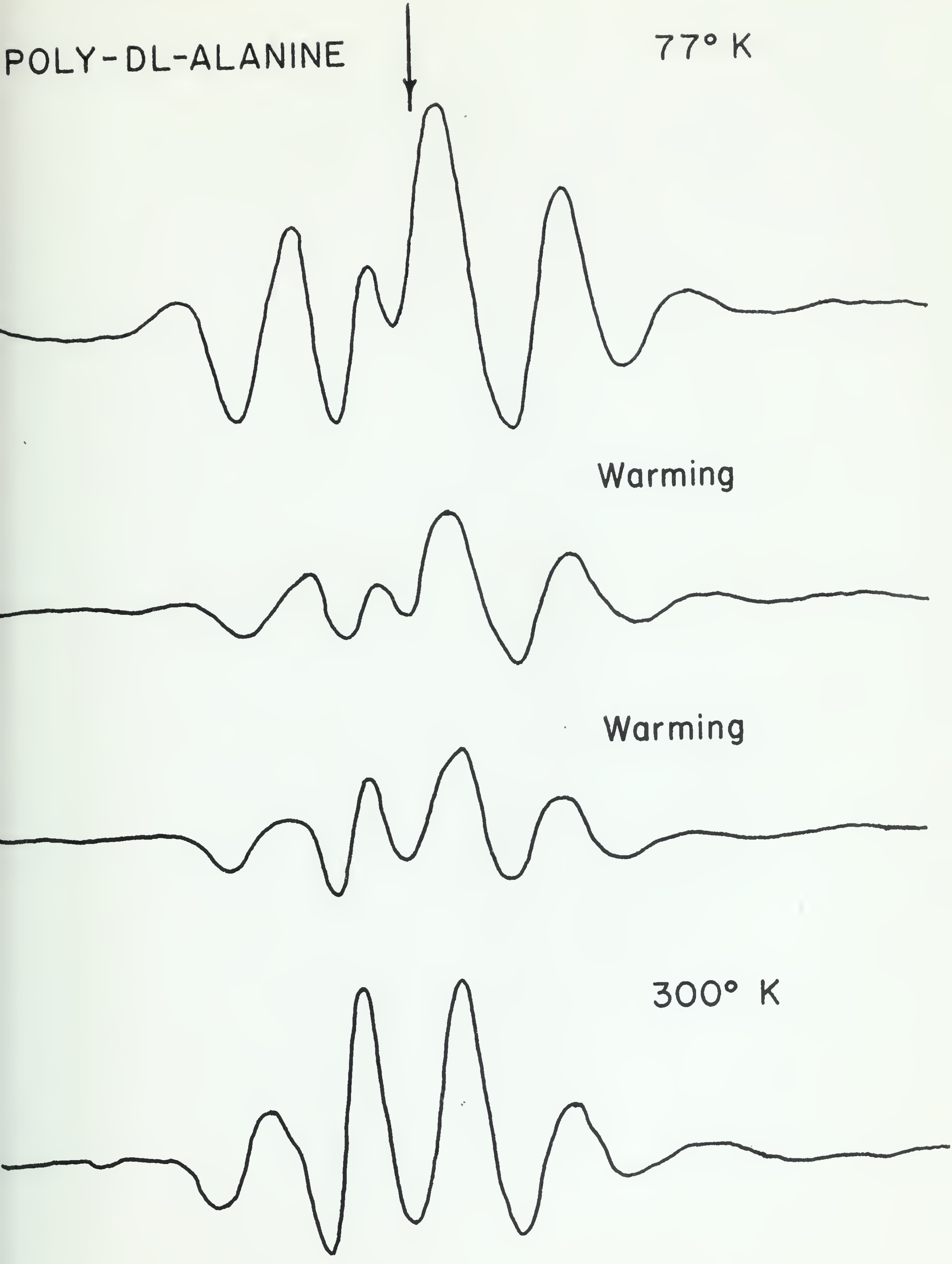
from the peak by ~11 gauss. The spacings of the components of the quintet are ~21 gauss. As in many of the resonances observed at 77°K, no plausible single radical can be identified here; apparently the pattern is the result of several superimposed radicals. Figure 10 illustrates the warming sequence typical of both poly-L-alanine and poly-DL-alanine. At 300°K, the typical room temperature pattern appears, with corresponding line intensities and splittings. The change is irreversible, i.e., no change in the pattern takes place when the temperature is lowered to 77°K again, indicating a definite chemical change in the radical from that which is originally observed at liquid nitrogen temperatures.

D. The Valine Group: Poly-L-Valine, Poly-L-Leucine, and Poly-DL-Leucine.

The structure of poly-L-leucine and poly-L-valine is quite similar. Both of the amino acids from which they are constructed possess side chains with two methyl groups at the ends, and exhibit similar chemical properties. Both amino acids are important in human metabolism and are listed as 'indispensable' to the diet. The molecular weights of the samples used were ~1,150 for poly-L-valine, ~3,500 for poly-L-leucine, and 4,500-10,000 for poly-DL-leucine, giving molecules of ~18, ~30, and 40-88 residues in length. End group effects will therefore play little if any role in the spectra observed.

At room temperature, the spectrum of poly-L-valine is essentially a singlet as shown in Figure 11; however, very low amplitude magnetic field modulation shows the existence of structure, i.e., the broad singlet appears actually to be an incompletely resolved doublet whose components are spaced ~5 gauss apart. When the sample is opened to air, an extremely rapid quenching of the spectra is observed (see Figure 12), the resonance becoming almost undetectable within three minutes' time. This may be contrasted to the 9 line spectra, unaffected by air reported for the simple amino acid, L-valine.

FIGURE 10. WARMING SEQUENCE: POLY-DL-ALANINE
This illustrates the migration of spin density to the room temperature configuration. Poly-L-alanine reacts in the same manner. (spectrometer gain is the same)


POLY-DL-ALANINE
77° K
Warming
Warming
300° K

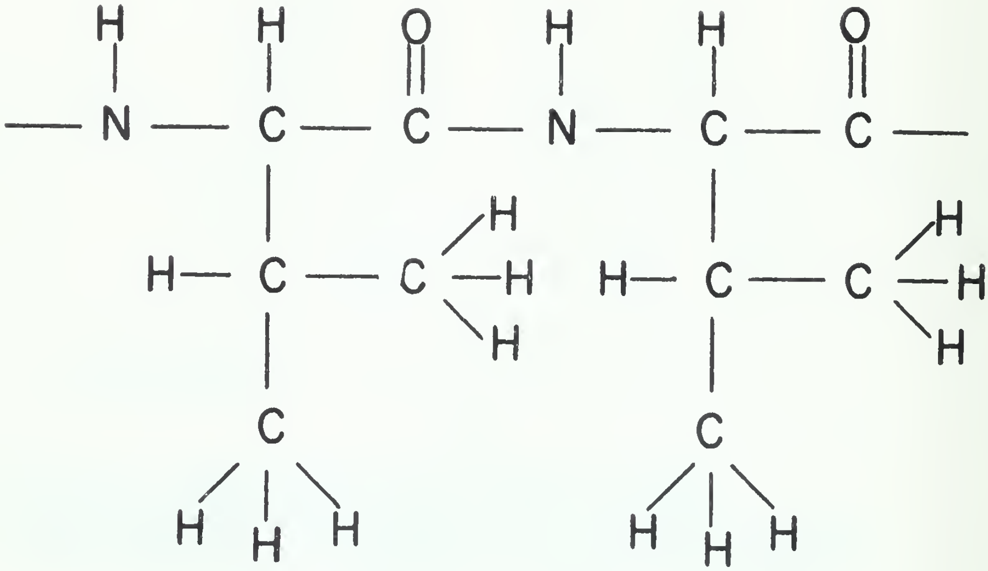


Fig. 11 (Above) Segment of a Poly-L-Valine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Valine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

POLY-L-VALINE

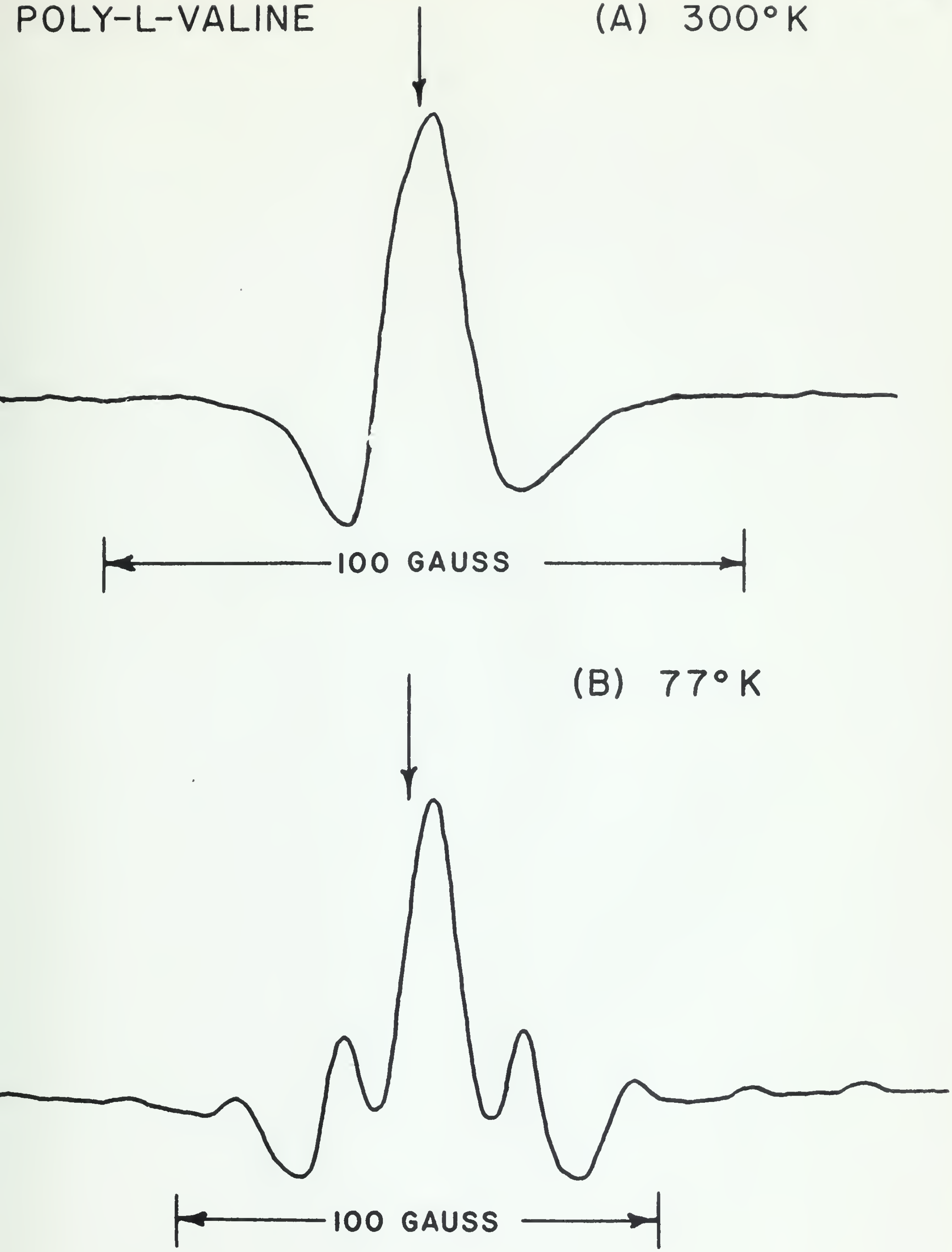
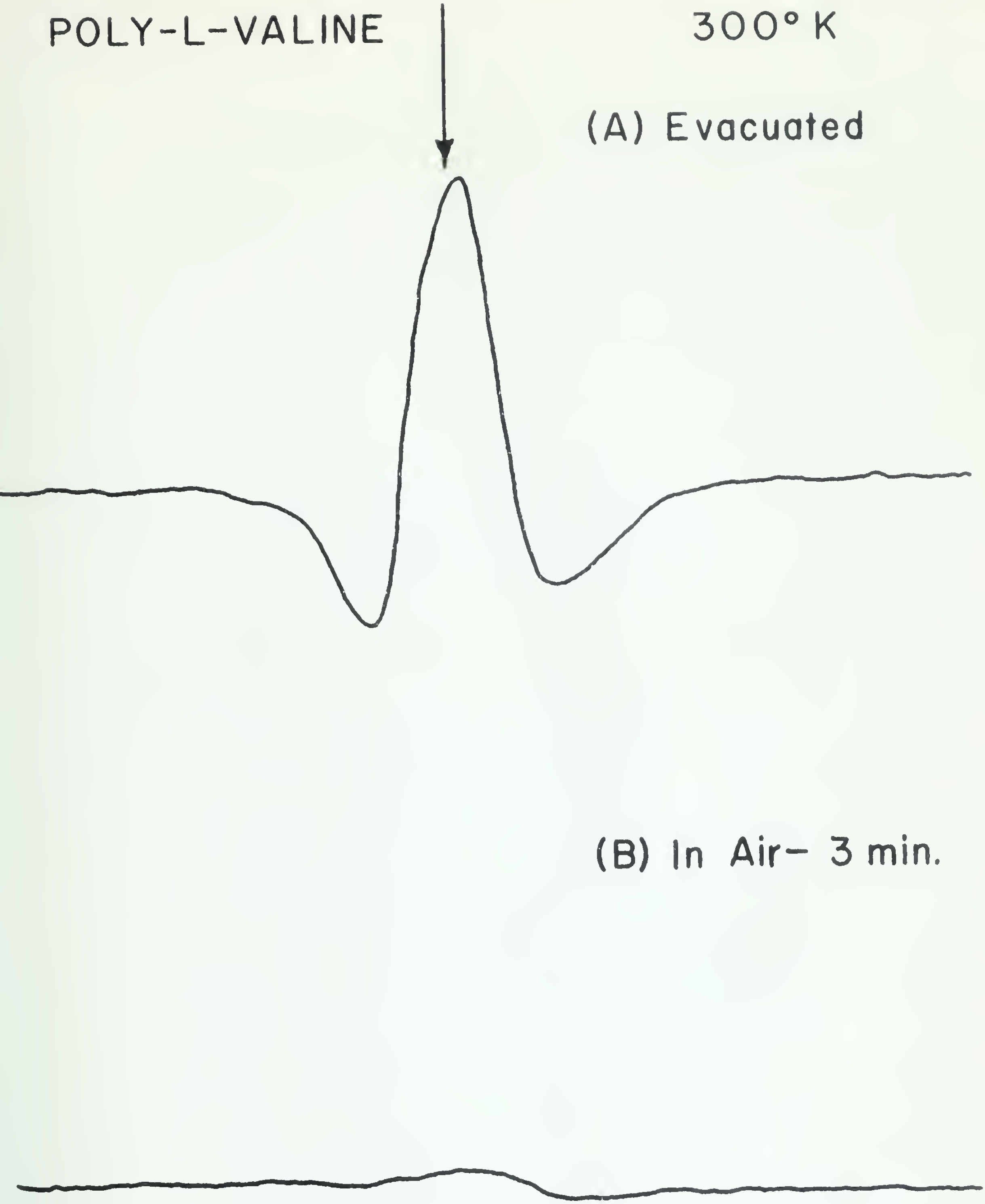


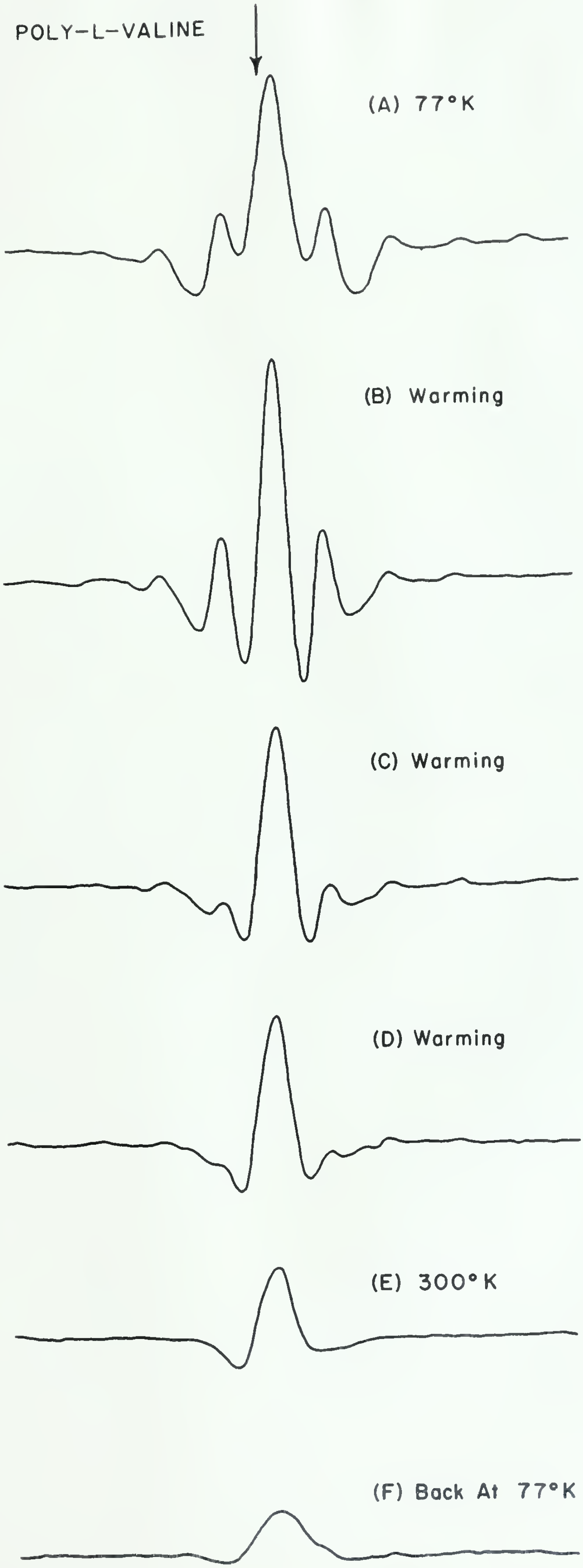
FIGURE 12. OPEN TO AIR SEQUENCE: POLY-L-VALINE
Gain settings are the same; time is measured from opening to air to the center of the resonance. Note the strong quenching effect for this radical.


POLY-L-VALINE
300° K
(A) Evacuated
(B) In Air– 3 min.

Poly-L-leucine and poly-DL-leucine display nearly identical spectra at 300°K and at 77°K. (See Figure 14 and Figure 15). The primary character of the room temperature curves is that of a strong doublet of $\sim$20 gauss splitting upon which is superimposed a rather weak and poorly resolved triplet. This might occur from a combination of two radicals, primarily one in which the R-group is removed, giving rise to the strong doublet, plus a small mixture of a radical in which the α-carbon proton is removed, and the unpaired spin density now interacts with the two nearest protons of the R group. Interactions with other protons in the R group are now likely to be too weak to be detectable. When the sample is opened to air, a fairly strong singlet replaces the characteristic resonance. The singlet decays at a moderate rate becoming undetectable in 2 to 4 hours. (The amino acid, L-leucine, is unaffected by air and displays a complex pattern at 300°K.) When irradiated and observed at 77°K, evacuated samples of the three polypeptides of this series exhibit spectra of a similar nature. All show a dominant single line, surrounded by six or eight smaller lines (the outer pair of lines are quite weak, and their presence is not definitely established) whose intensities do not fit the ratios expected for equal or nearly equal coupling with six (or eight) protons.

When allowed to warm to 300°K, the spectra of all three compounds gradually changed to the pattern which is observed for a sample irradiated at 300°K, an example of which is shown in Figure 13 for poly-L-valine. Of interest is the change from (A) to (B) in the figure, illustrating the effect on the sharpness of the lines when the anisotropic broadening from hindered rotations at 77°K is removed by warming. Gradual migration of the spin density may be followed in (C) through (E) coupled with a loss in detection efficiency as predicted in equation 13. In (E), the characteristic room

FIGURE 13. WARMING SEQUENCE: POLY-L-VALINE
Spectrometer gain is the same for all spectra, (A)-(F).


POLY-L-VALINE
(A) 77°K
(B) Warming
(C) Warming
(D) Warming
(E) 300°K
(F) Back At 77°K

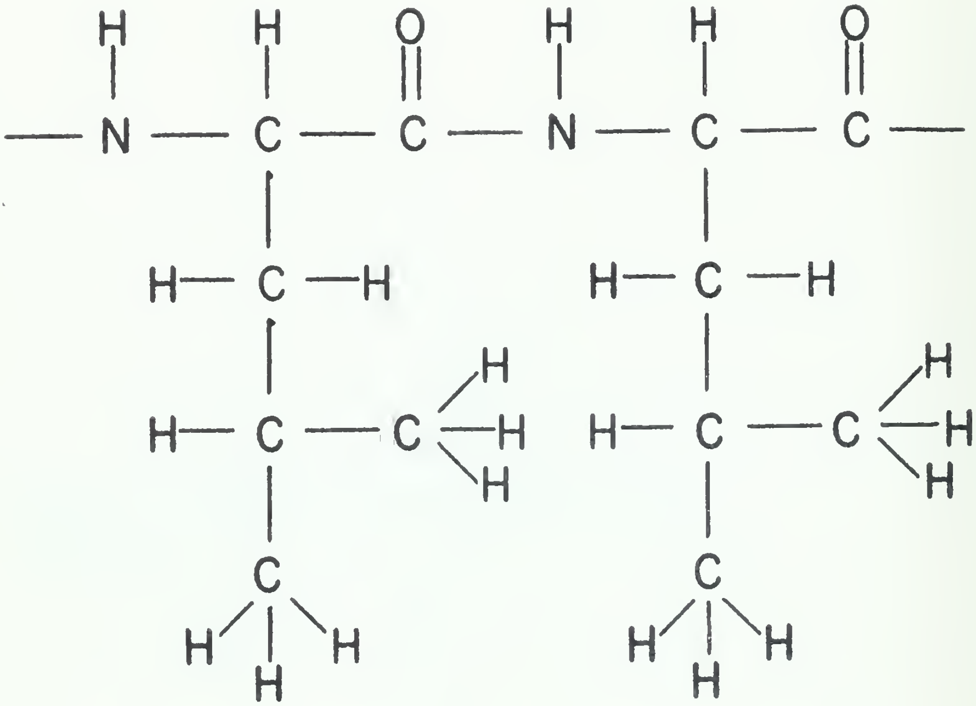
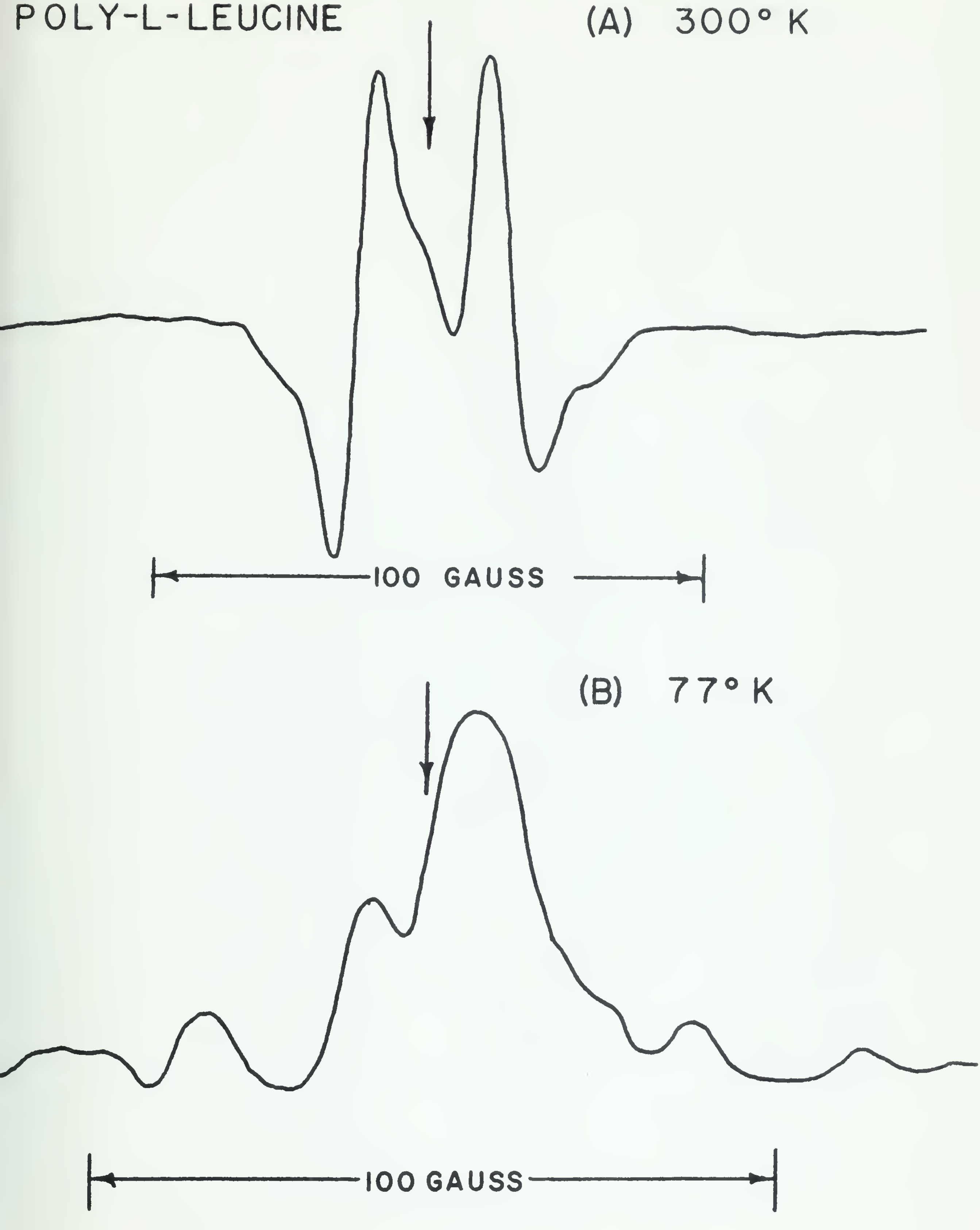


Fig. 14 (Above) Segment of a Poly-L-Leucine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Leucine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)


POLY-L-LEUCINE
(A) 300° K
100 GAUSS
(B) 77° K
100 GAUSS

[illegible]

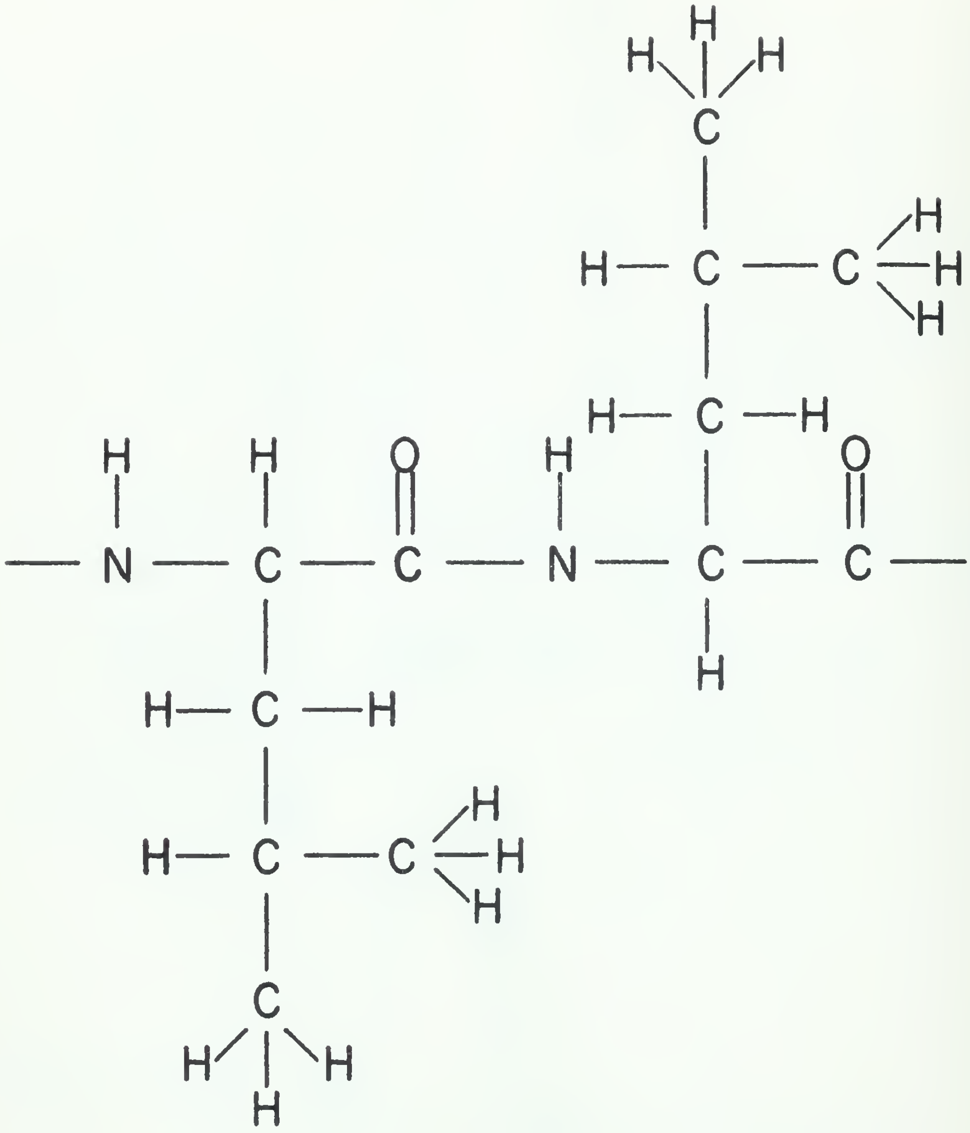


Fig. 15 (Above) Segment of a Poly-DL-Leucine molecule: (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-DL-Leucine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

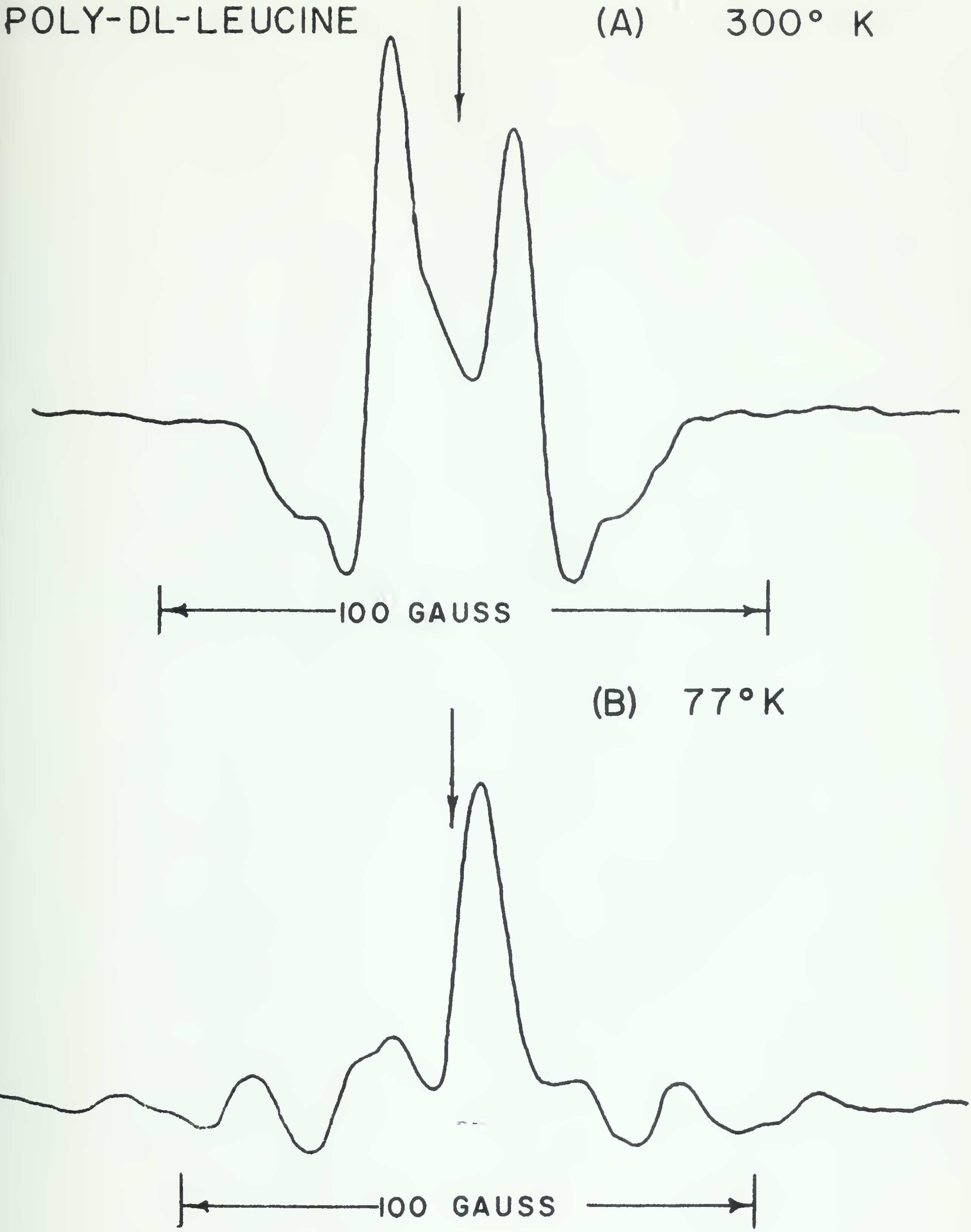
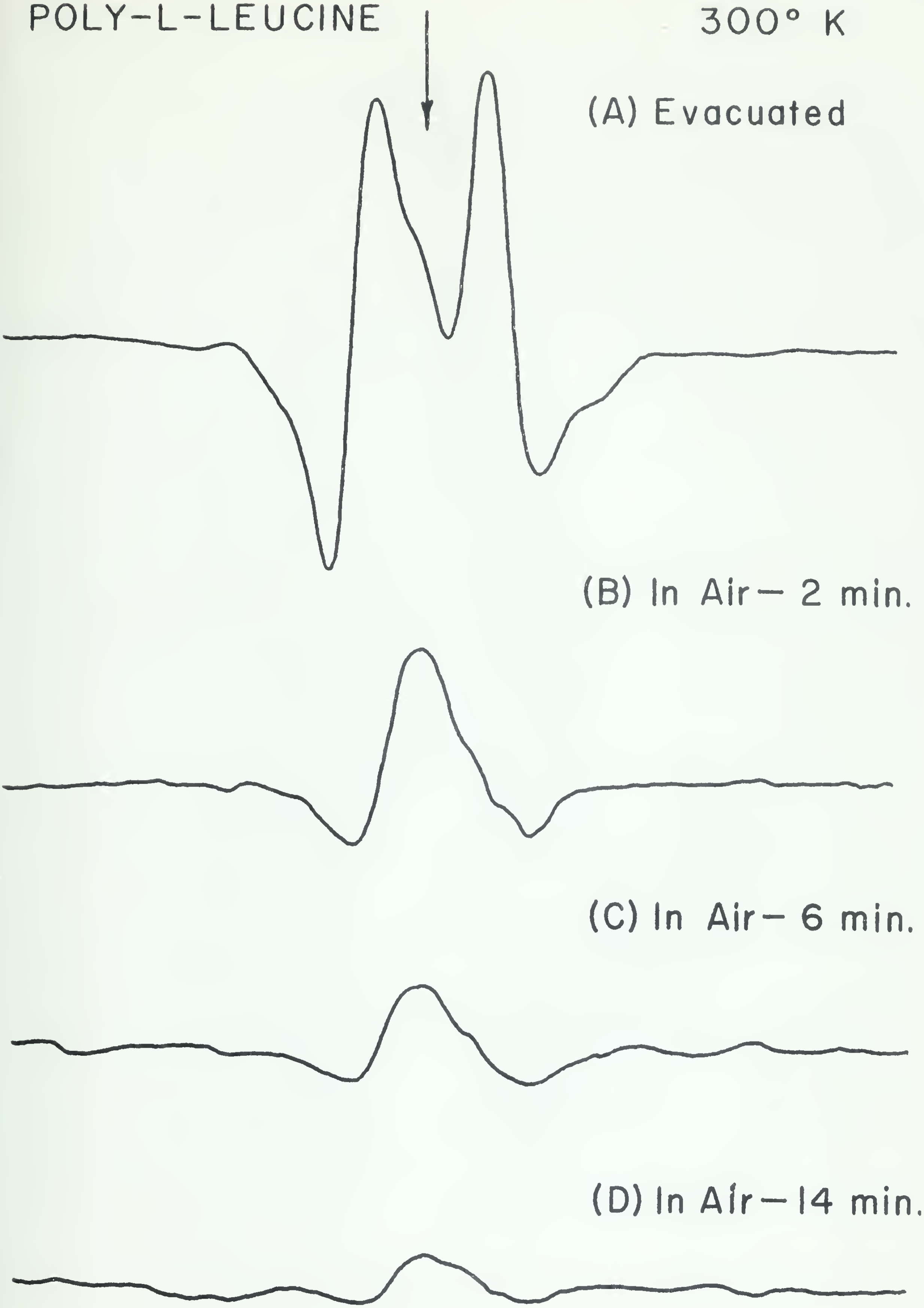

POLY-DL-LEUCINE
(A) 300° K
100 GAUSS
(B) 77°K
100 GAUSS

FIGURE 16. OPEN TO AIR SEQUENCE: POLY-L-LEUCINE
This sequence shows the moderately rapid quenching of O_2, and the subsequent decay of the singlet produced.

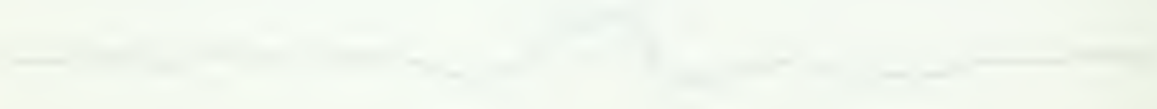

POLY-L-LEUCINE
300° K
(A) Evacuated
(B) In Air – 2 min.
(C) In Air – 6 min.
(D) In Air – 14 min.

[illegible]

[illegible]

[illegible]

[illegible]

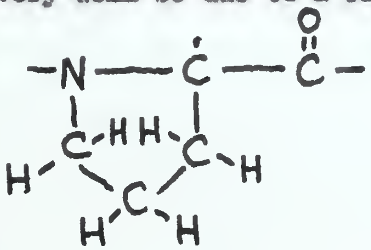


temperature spectrum is finally reached, and then the spectrum obtained after returning the sample to 77°K is displayed in (F). The broadening and reduction in intensity of the resonance in (F) is typical of the effect of anisotropic broadening at this temperature. Similar effects are noted for the other members of this group.

E. The Proline Group: Poly-L-Proline, Poly-Hydroxy-L-Proline, and Poly-O.Acetyl-Hydroxy-L-Proline. Availability of the members of this group allows the investigation of the effect of substitutions in the basic R-group structure on the resonances observed. Segments of the polypeptide chains and the spectra obtained are illustrated in Figures 17-19.

The basic residue of the group, the amino acid, proline, is widely distributed among the proteins, with an especially large proportion in zein and gelatin, while hydroxy-L-proline has a rather limited distribution except for a large amount in gelatin and collogen. O.acetyl hydroxy-L-proline is an artificially substituted derivative of proline and is not found naturally. Minimum chain length for this group is 26 residues, and therefore end groups will have little influence.

Curves for all three compounds show an expected similarity, both at 300°K and at 77°K, the basic characteristic of all being a five line spectrum of varying line intensities and spacings. For poly-L-proline, the existence of what apparently is a weak pair of lines outside the central triplet (see Figure 17) is in doubt. Repeated sweeps through the resonance did not consistently reproduce the pattern shown; however, a small variation in spectrometer sensitivity could easily mask such weak components. The central triplet may very well be due to a radical of the form



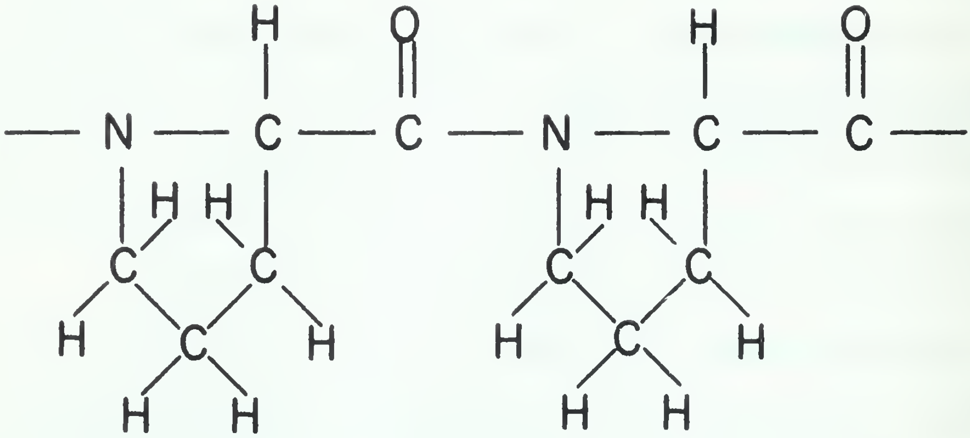
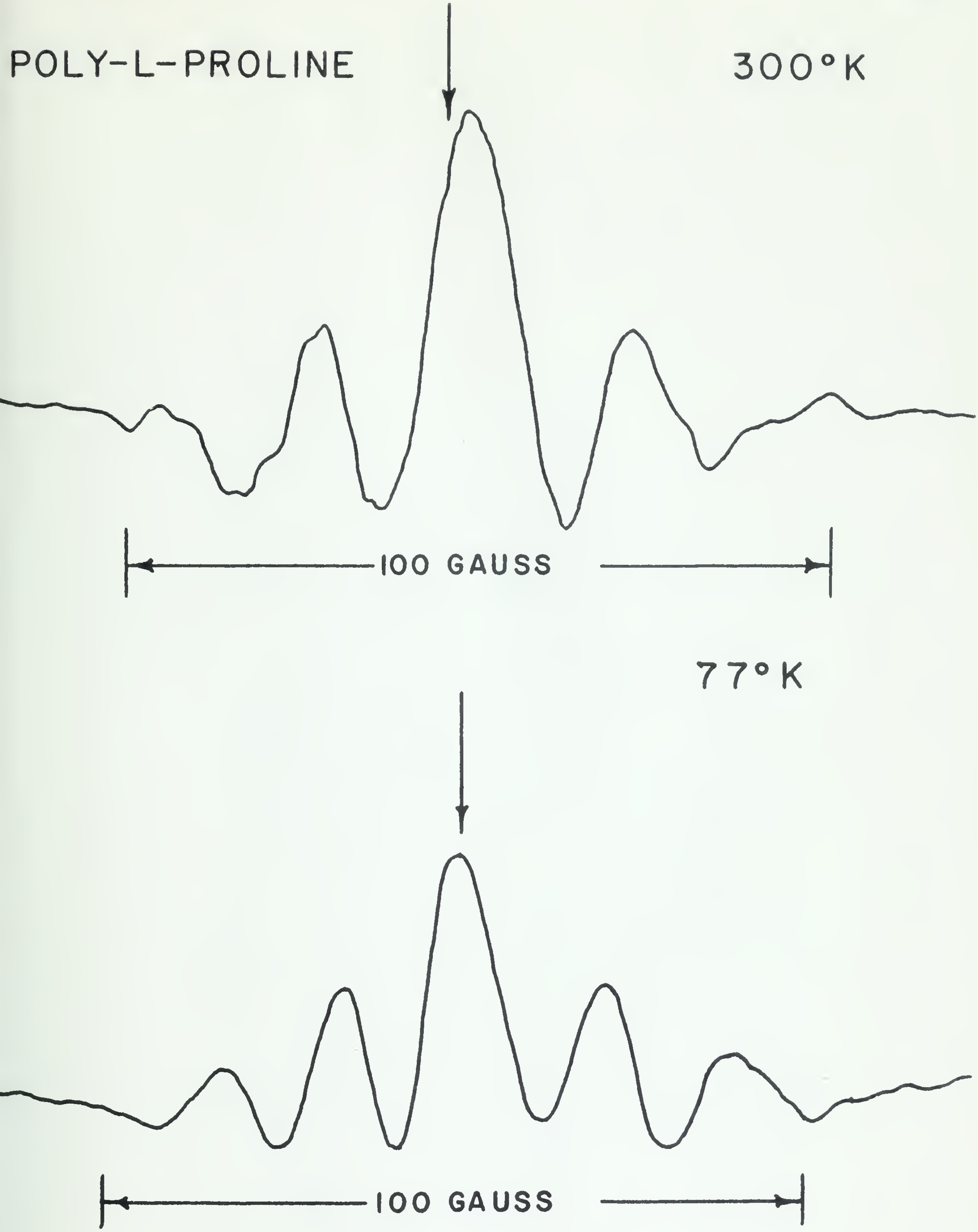


Fig. 17 (Above) Segment of a Poly-L-Proline molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Proline: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)


POLY-L-PROLINE
300°K
100 GAUSS
77°K
100 GAUSS

[illegible]

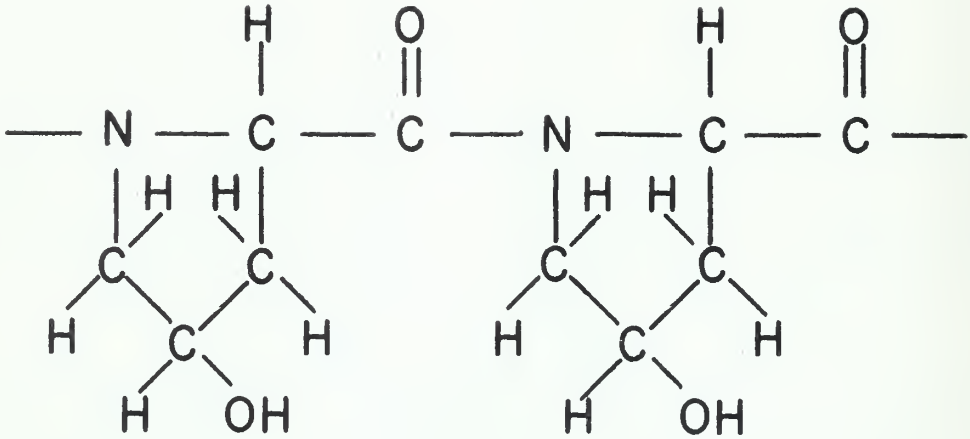
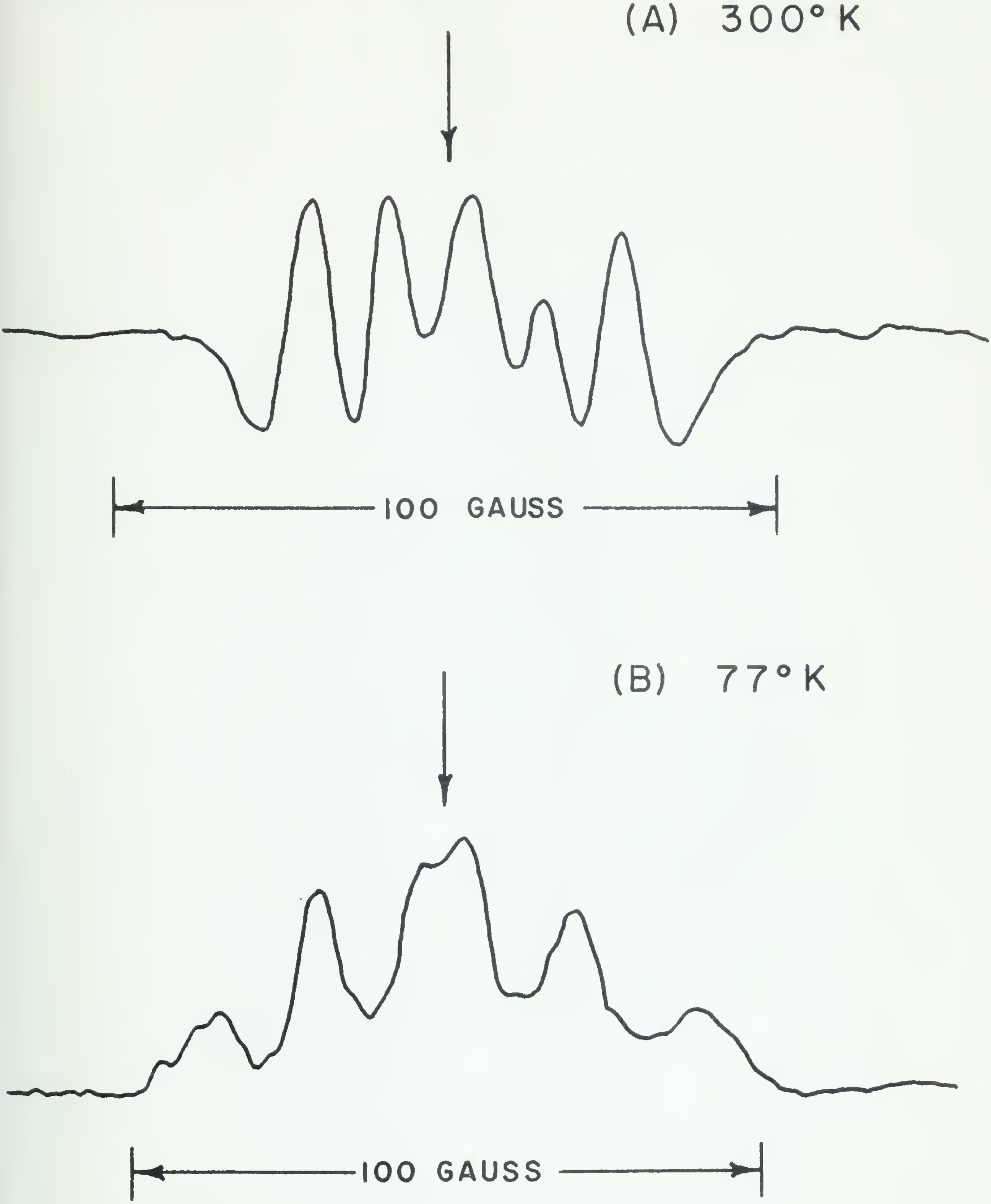


Fig. 18 (Above) Segment of a Poly-Hydroxy-L-Proline molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-Hydroxy-L-Proline: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

POLY-HYDROXY-L-PROLINE



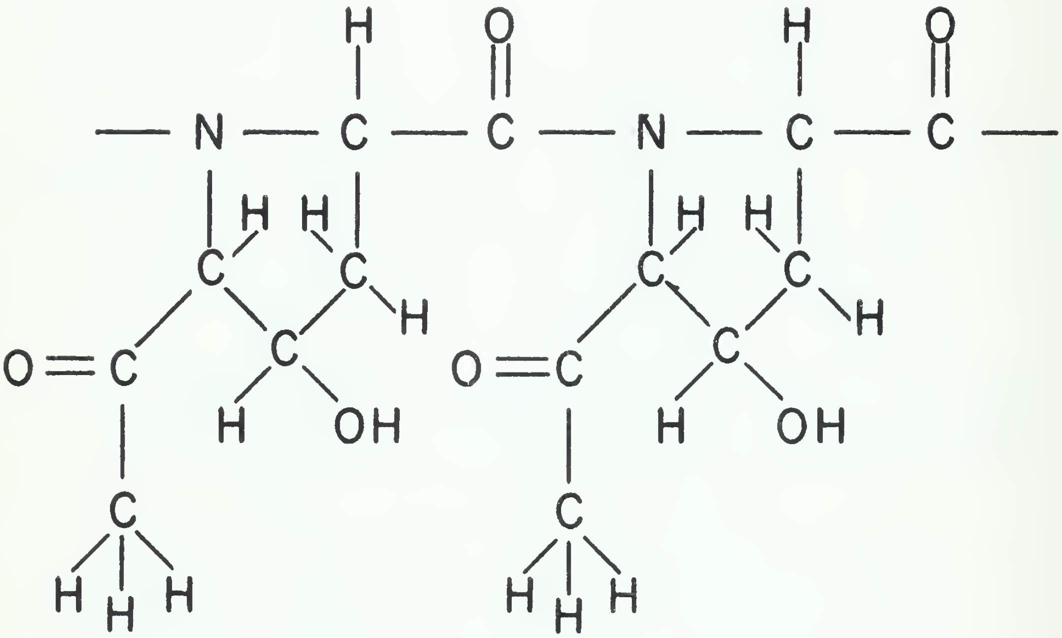
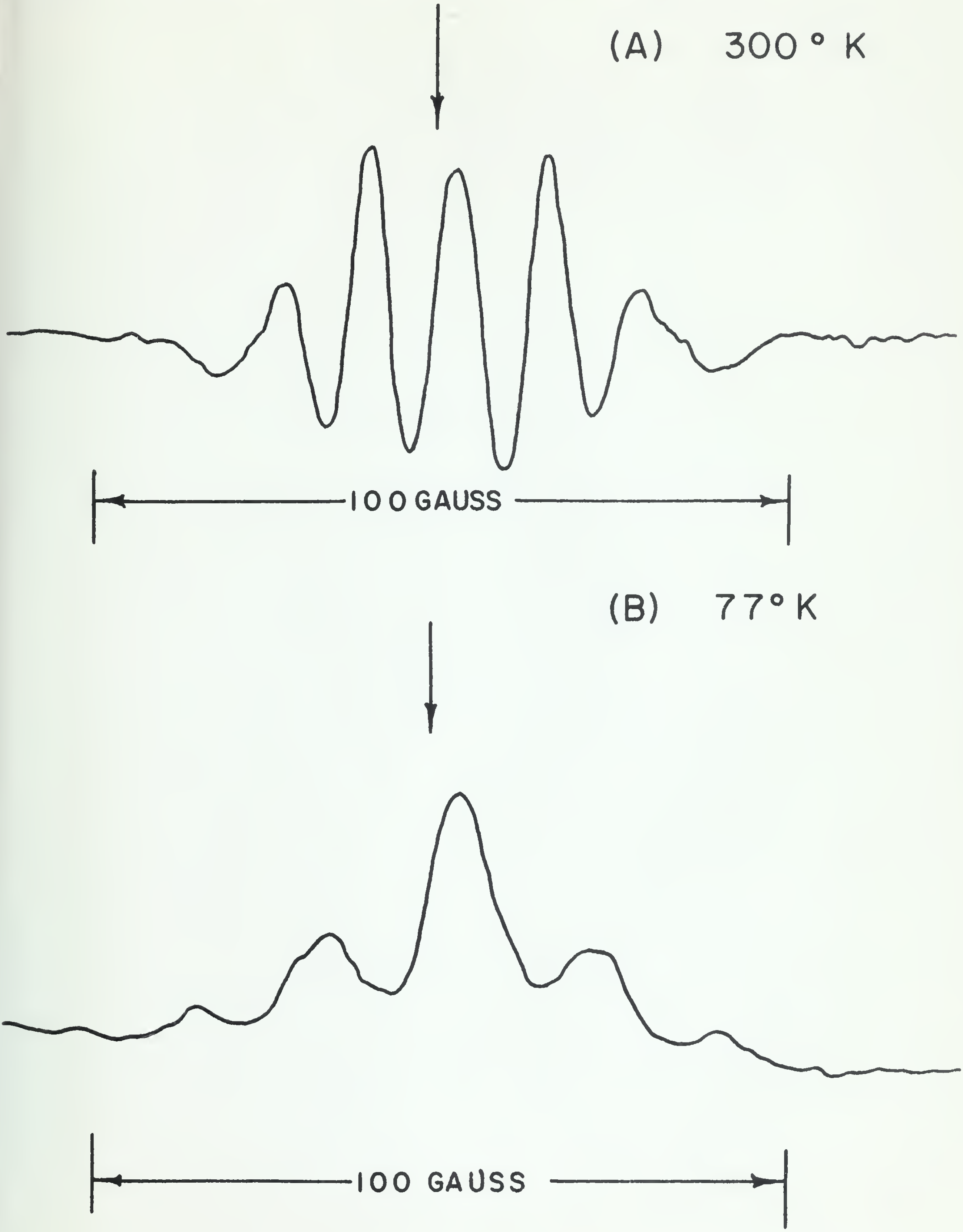


Fig. 19 (Above) Segment of a Poly-O. Acetyl Hyroxy-L-Proline molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-O. Acetyl Hyroxy-L-Proline: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

POLY-O.ACETYL-HYDROXY-L-PROLINE



where the unpaired spin density interacts quite strongly with the two protons on the closest carbon through hyperconjugation. Interaction with the other protons would exist, but only very weakly from a small overlapping spin density on the adjoining carbons, giving rise to the extremely weak components observed.

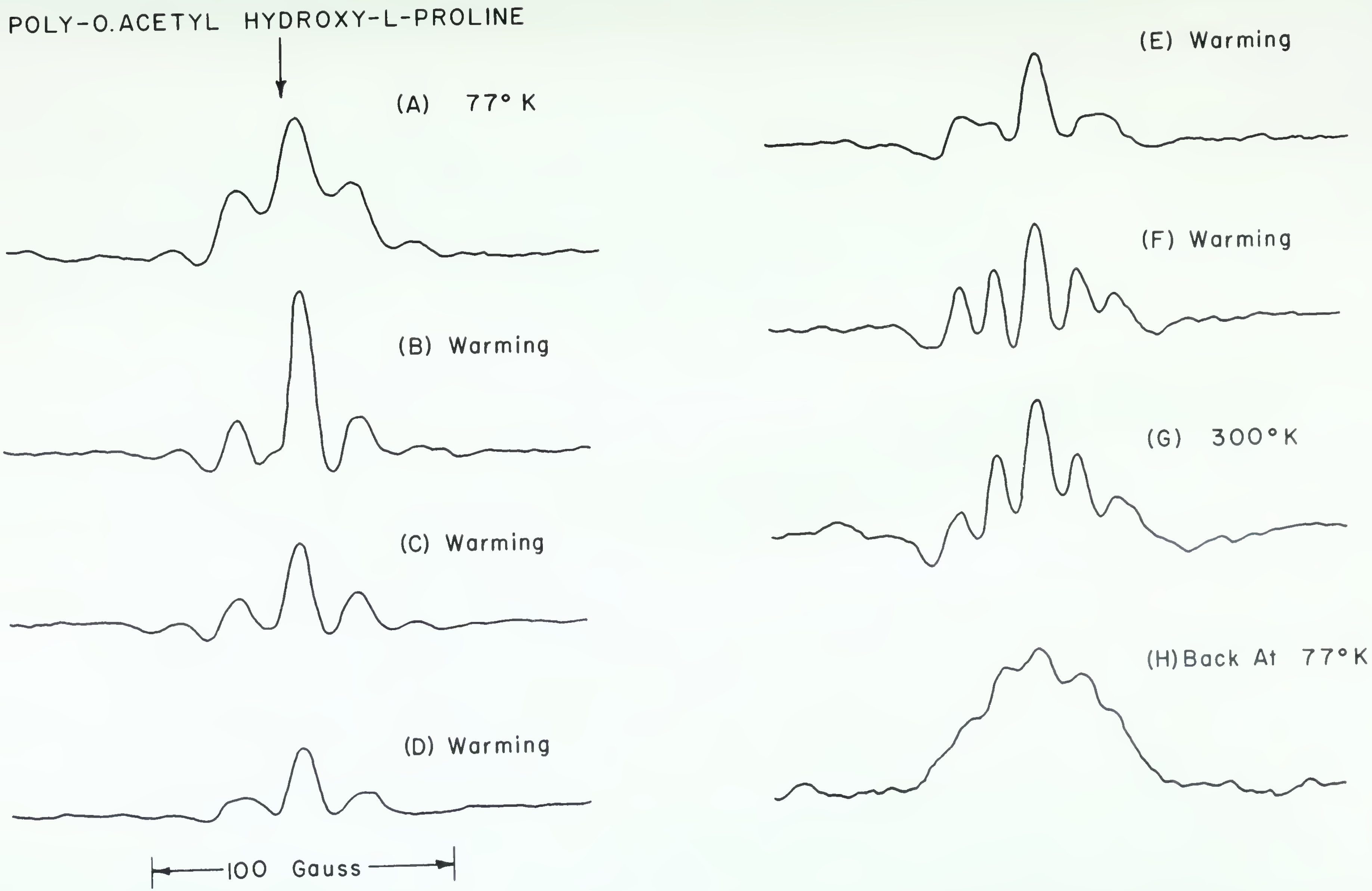
The other two members of this series both display five line resonances at 300°K which in this case have component splittings of ~12 gauss, roughly half that for proline. This probably involves a combination of unequal coupling with one and then two protons, but identification of the possible radicals will not be attempted.

An interesting comparison between these compounds may be derived from the effect of oxygen on the respective radicals. Both poly-L-proline and poly-O.acetyl-hydroxy-L-proline show a strong quenching of the resonance, weak singlets appearing within 4 minutes, which then decay. The spectrum of poly-hydroxy-L-proline, however, remains unchanged when opened to air, but begins decaying slowly and gradually disappears in 24 hours. Thus there appears to be a stabilizing or protecting effect due to the addition of the hydroxyl (OH) group to proline, which then is cancelled or counteracted with the addition of the acetyl group.

When warmed, the poly-L-proline and poly-hydroxy-L-proline samples both change to the triplet characteristic of proline, while the rather unusual warming cycle for poly-O.acetyl hydroxy-L-proline is shown in Figure 20. The effects of hindered rotation, spin density migration, and dipole-dipole broadening are apparent.

The Ringed Group: Poly-L-Phenylalanine, Poly-DL-Phenylalanine, Poly-L-Tyrosine, Poly-L-Tryptophan, and Poly-DL-Tryptophan. The members of this group, phenylalanine, tyrosine, and tryptophan, are found as a result of the

FIGURE 20. WARMING SEQUENCE: POLY-O.ACETYL-HYDROXY-L-PROLINE This sequence shows the gradual development of the room temperature pattern. In (H) the effect of anisotropic broadening is readily apparent.

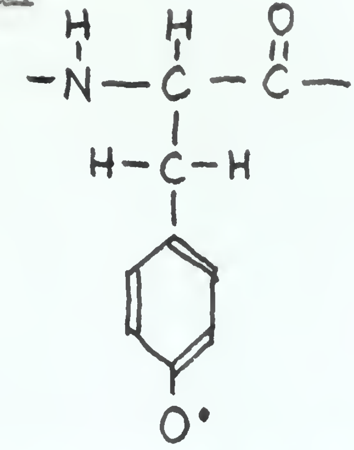

POLY-O.ACETYL HYDROXY-L-PROLINE
(A) 77° K
(B) Warming
(C) Warming
(D) Warming
100 Gauss
(E) Warming
(F) Warming
(G) 300° K
(H) Back At 77° K

hydrolysis of a wide variety of proteins, although usually in rather small amounts. All are characterized by the presence of an aromatic ring as part of their R-group structure. Average molecular weights of the samples used were such that a minimum of 21 residues was present in the smallest molecules, and hence end group effects were negligible.

In early work on the amino acids of this group,[51] the broad single resonance of roughly 25 gauss spread, which would be expected for an unpaired spin density on the ring structure, was observed for both tyrosine and tryptophan, while phenylalanine gave a similar broad resonance superimposed on a triplet structure. In the polypeptides formed from these compounds this behavior seems to be the same (see Figures 21, 22, 25, 26, and 27), with the exception of the phenylalanines. In these two compounds, the spectrum at 300°K now appears to be a strong broad resonance resulting from a superposition of a doublet of ~17 gauss splitting and, quite possibly, a broad triplet or singlet. This may occur as a result of breaking the C-C bond to the R group, leaving an unpaired electron to interact with the single remaining proton on the polypeptide chain backbone in a radical similar to poly-glycine and giving the doublet with ~17 gauss splitting. The additional broadening with an apparent center line would then arise from an unpaired electron stabilized on the ring structure and interacting only weakly with the attached CH_2 group. This radical structure shows a strong oxygen effect (Figure 24), the resonance disappearing within three minutes after being opened to air.

In the polypeptides of tyrosine and tryptophan the resonances observed at room temperature were extremely weak and short-lived even in a vacuum. In poly-L-tyrosine, it seems probable that the proton from the OH group would

be lost to form the radical



in which the unpaired electron would be delocalized in the π -orbital system of the ring structure. Hyperfine splitting from the protons on the ring would take place through the mechanism of configurational interaction; however, splittings of ~2 gauss would be expected[52], and these would only cause an unresolvable broadening for these polycrystalline samples.

Similar spectra are observed for poly-L-tryptophan and poly-DL-tryptophan. Loss of the NH proton is expected for these compounds. This would produce a radical that would allow the unpaired spin to become delocalized on the two ring structures which are basically co-planar and thus would have overlapping π orbital systems.

When the sample is opened to air, (see Figure 28) the broad singlet appears to become enhanced. This unusual behavior may be explained by a consideration of the characteristics of the spectrometer system. In normal operation, low amplitude magnetic field modulation is employed for maximum resolution of any closely spaced hyperfine splittings. This, however, lowers the relative intensity of broad resonances. In this case, the following sequence of events explains the effects seen in Figure 28. The broad resonance in (A), observed for the sample when irradiated in a vacuum, is replaced by a much narrower oxygen singlet when opened to air. This narrow line appears

stronger, (B), because of the effects of field modulation. In (C) and (D), the normal decay of the oxygen singlet is observed. Unfortunately, the short lifetime of the resonance in poly-L-tyrosine in a vacuum prevented the observation of any similar oxygen effect for it.

At 77°K, the tryptophan and tyrosine samples again show the broad single resonance, with slightly greater intensity. This indicates that migration of charge may occur in these molecules even at this temperature. A contributing factor to this ease in migration undoubtedly is the high degree of planarity in the R group, resulting in overlapping π-orbitals. When the sample is warmed, no significant changes take place; the resonances decrease in intensity, and rather quickly disappear. Lowering the temperature to 77°K again does not make the resonance detectable, i.e., a definite decay of the radicals has occurred.

For poly-L-phenylalanine and poly-DL-phenylalanine, a representative warming cycle is shown in Figure 23. Absence of the easily removed proton of the OH group found in tyrosine alters the behavior of these samples both at 300°K and at 77°K. Here, at 77°K, a broad quintet gradually changes to the singlet characteristic of an unpaired electron on an aromatic ring structure.

G. The Acid Group: Poly-L-Aspartic Acid and Poly-L-Glutamic Acid. The members of this group are unique among the polypeptides studied since the side chain or R group is terminated with a carboxyl group (COOH). Both amino acids are present in large amounts in plant proteins, while glutamic acid is of particular importance in the metabolic processes of plants, animals, and micro-organisms. With average molecular weights of 7,000-8,500 and 15,000-20,000, corresponding to chains of 61-74 residues for poly-L-aspartic acid and of 116-155 residues for poly-L-glutamic acid respectively,

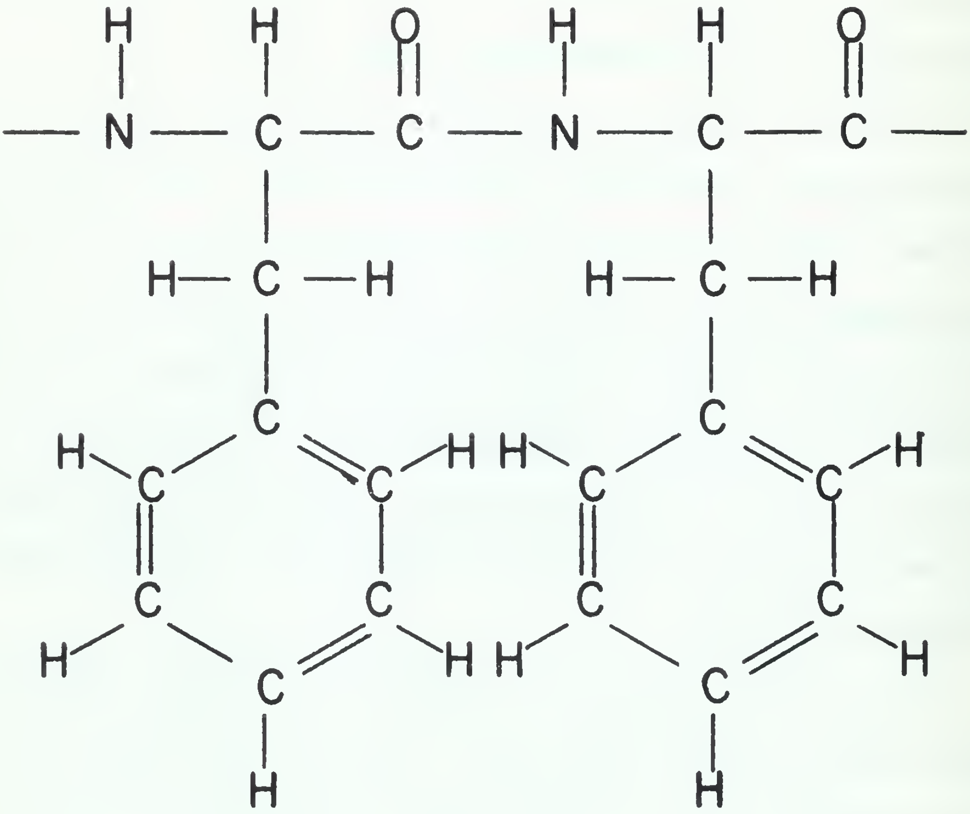
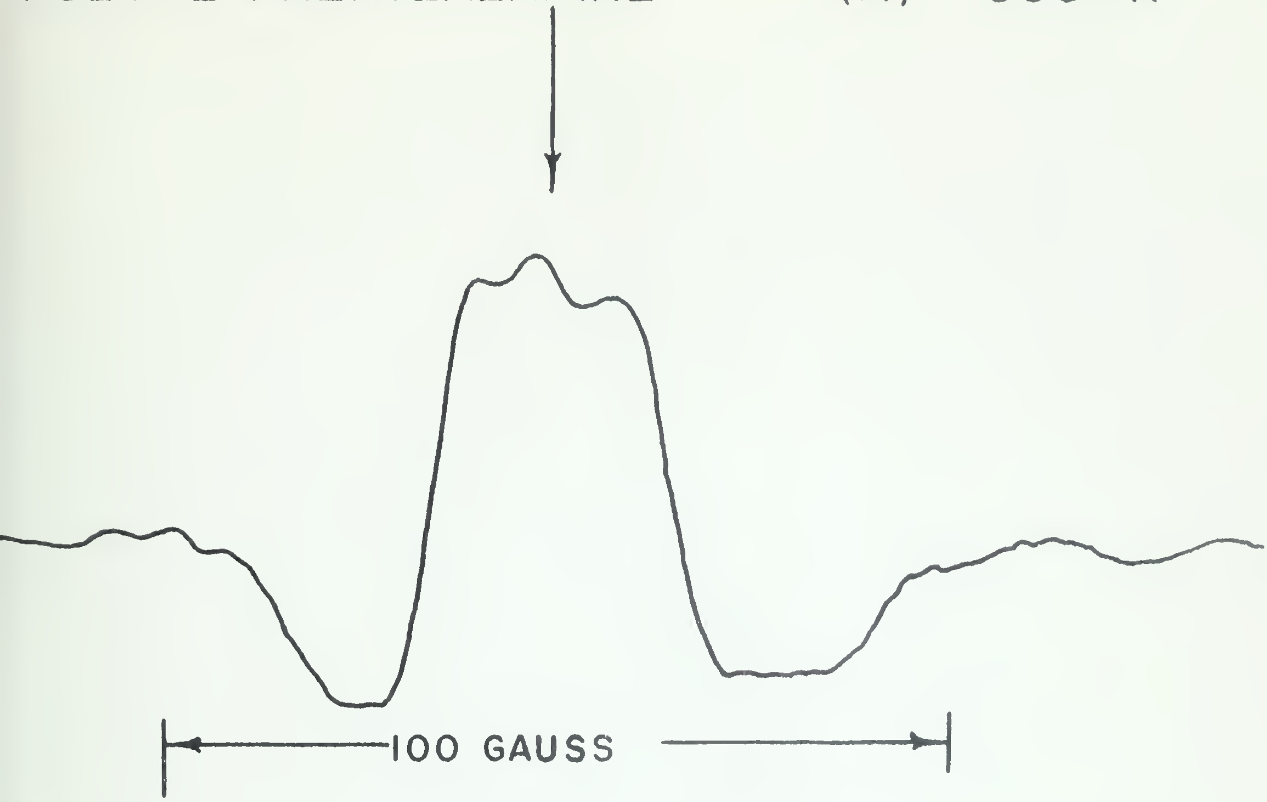
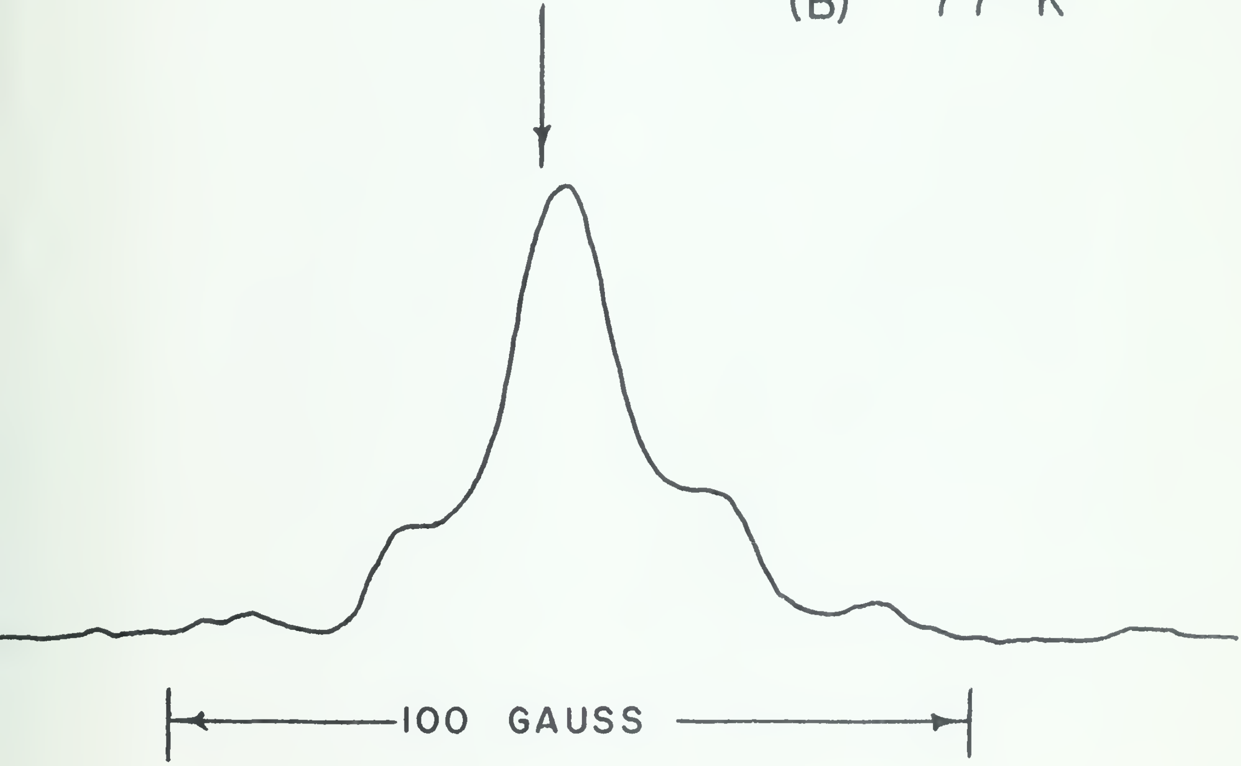


Fig. 21 (Above) Segment of a Poly-L-Phenylalanine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Phenylalanine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

POLY-L-PHENYLALANINE (A) 300° K



(B) 77° K



[illegible]

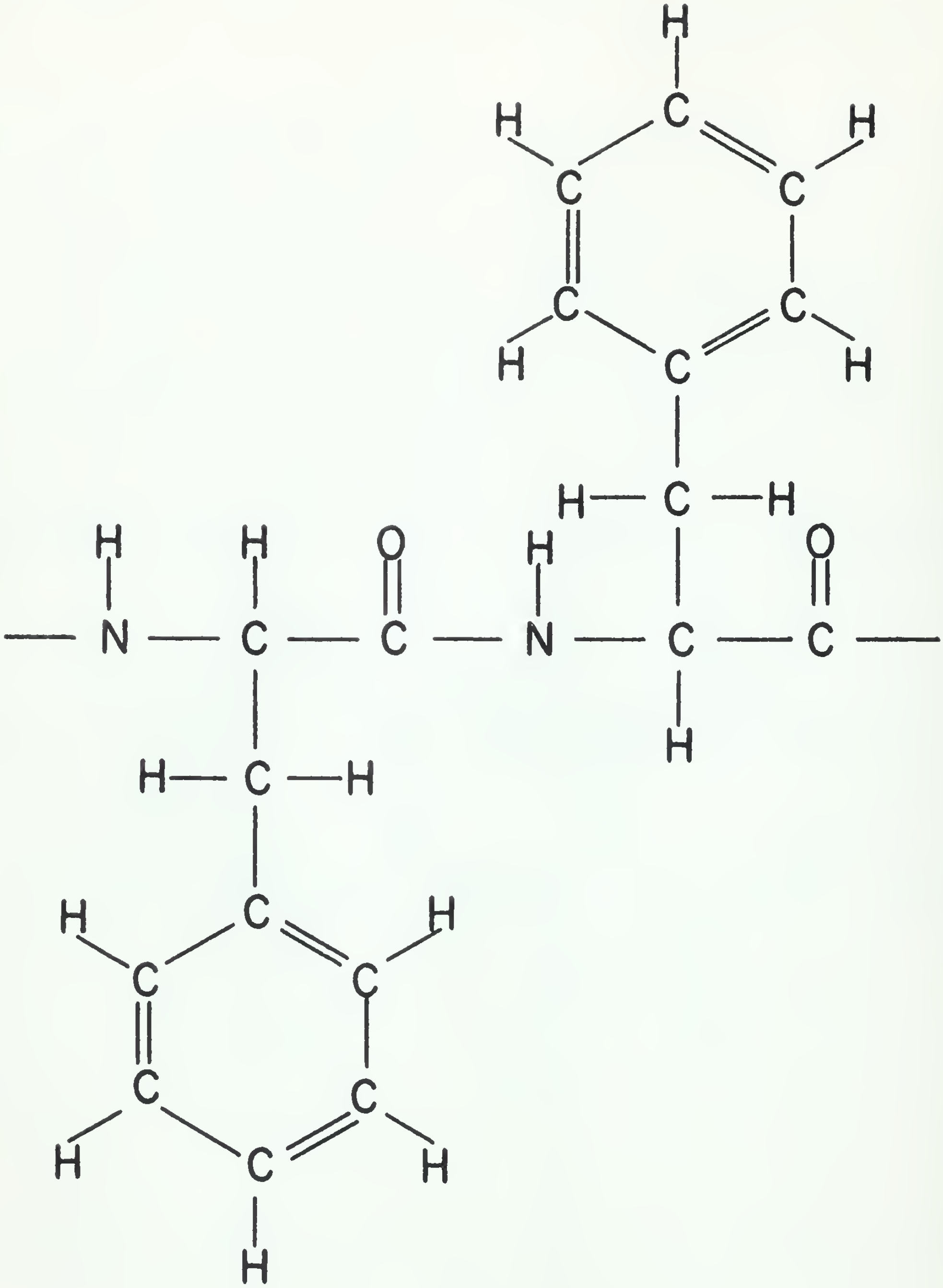


Fig. 22 (Above) Segment of a Poly-DL-Phenylalanine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-DL-Phenylalanine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

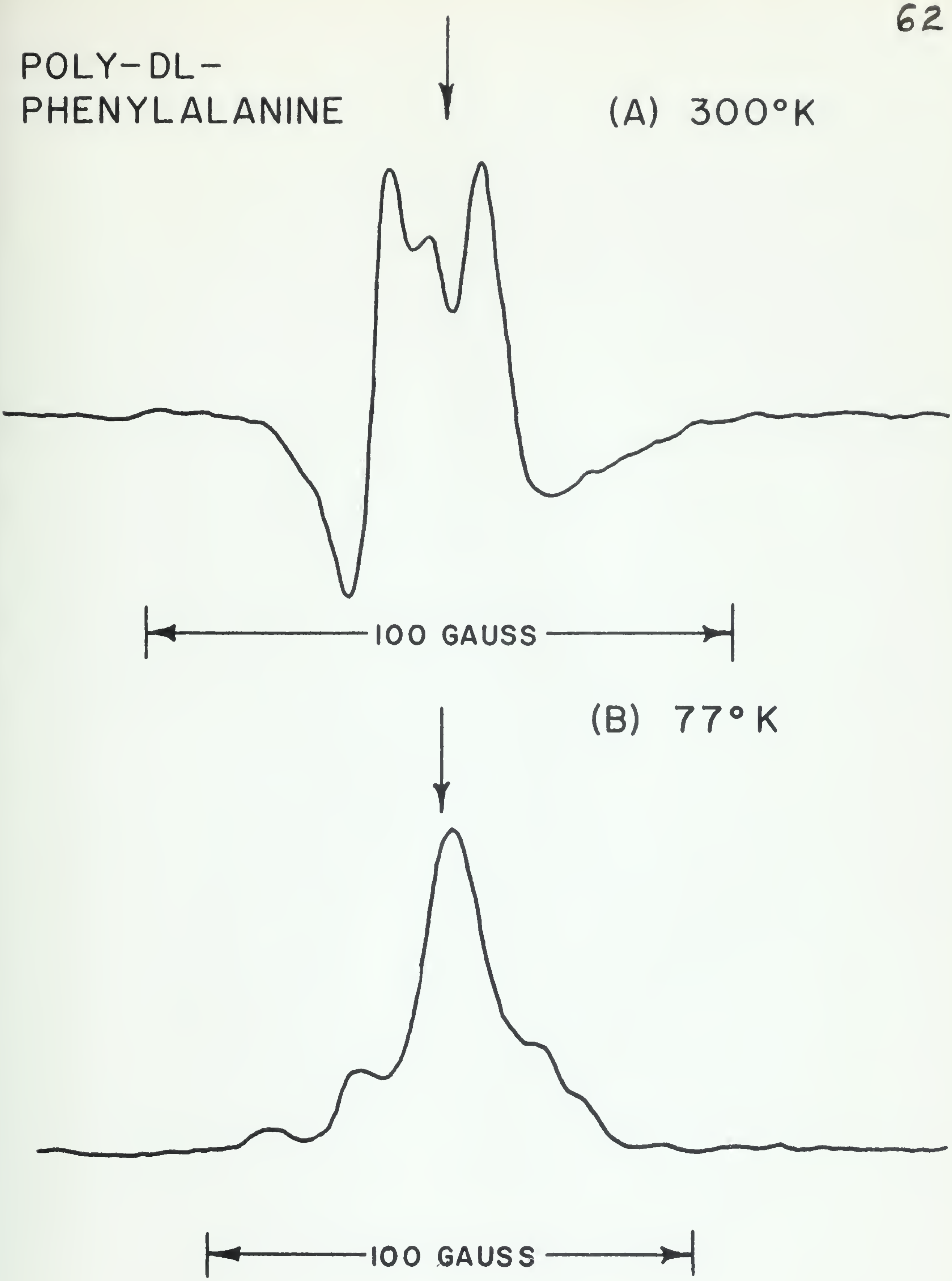

POLY-DL-PHENYLALANINE
(A) 300°K
100 GAUSS
(B) 77° K
100 GAUSS

FIGURE 23. WARMING SEQUENCE: POLY-L-PHENYLALANINE
Spectrometer gain is the same, (A)-(E).

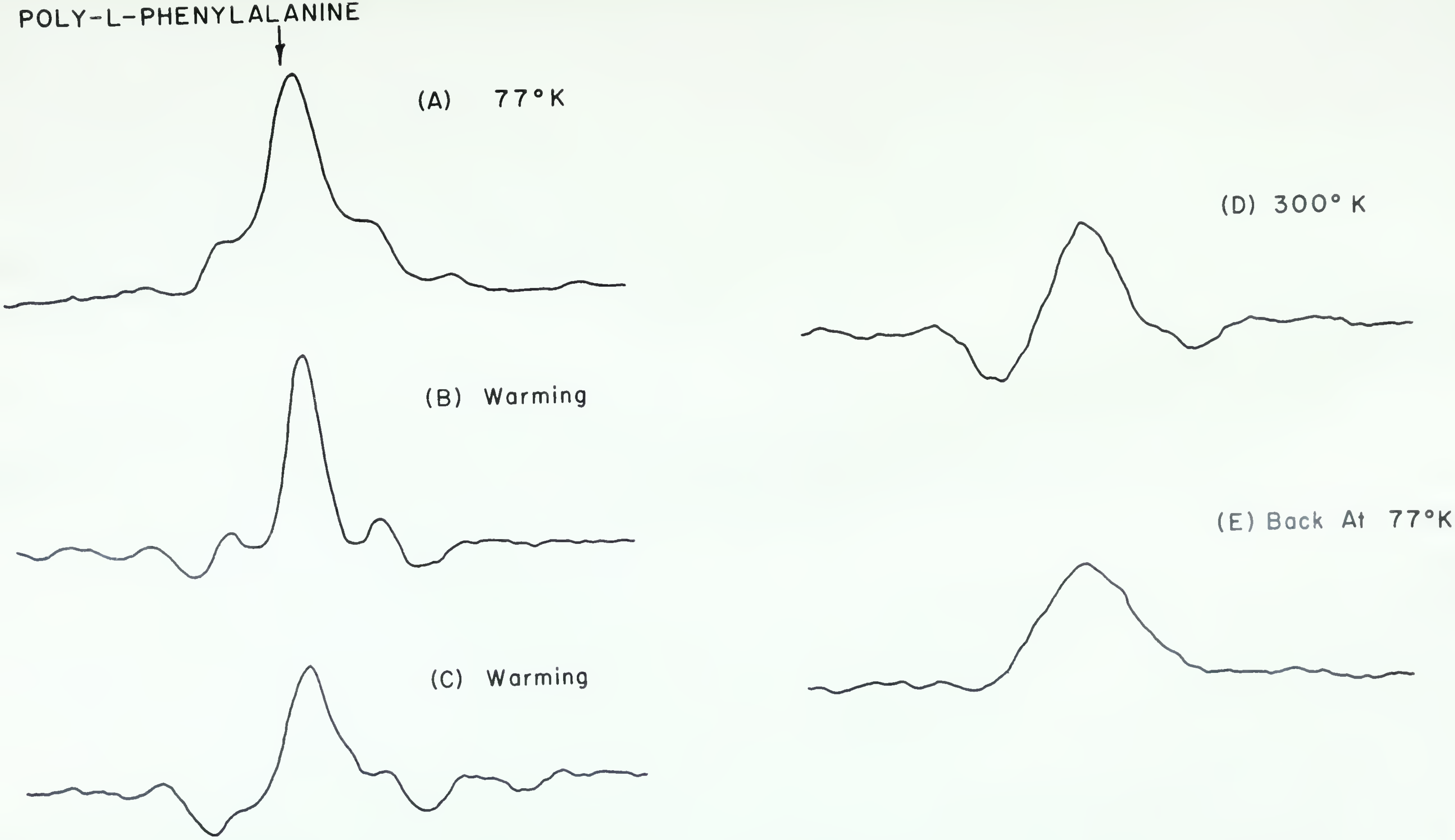
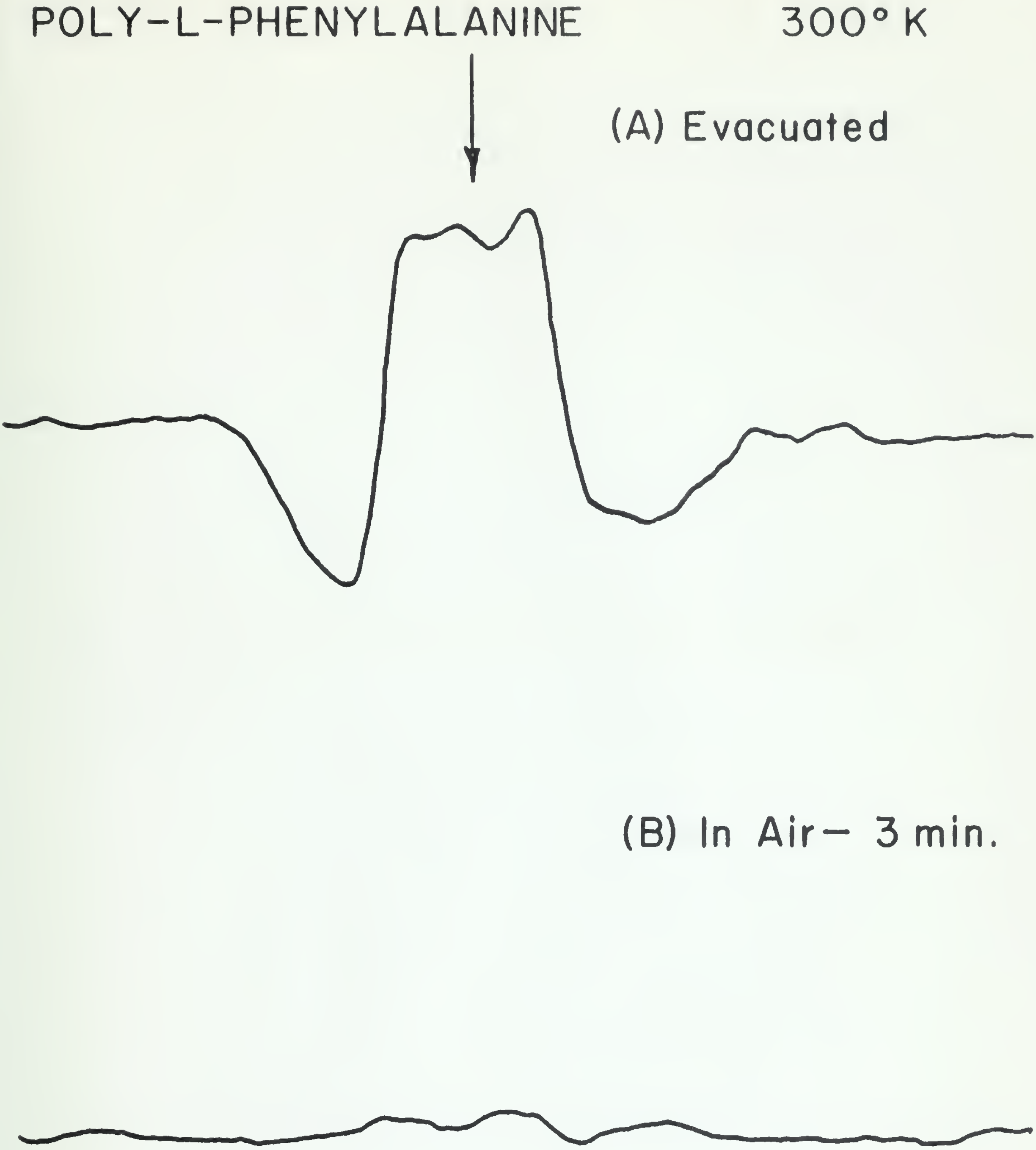

POLY-L-PHENYLALANINE
(A) 77°K
(B) Warming
(C) Warming
(D) 300° K
(E) Back At 77°K

FIGURE 24. OPEN TO AIR SEQUENCE: POLY-L-PHENYLALANINE
Spectrometer gain is the same in (A) and (B).


POLY-L-PHENYLALANINE
300° K
(A) Evacuated
(B) In Air – 3 min.

[illegible]

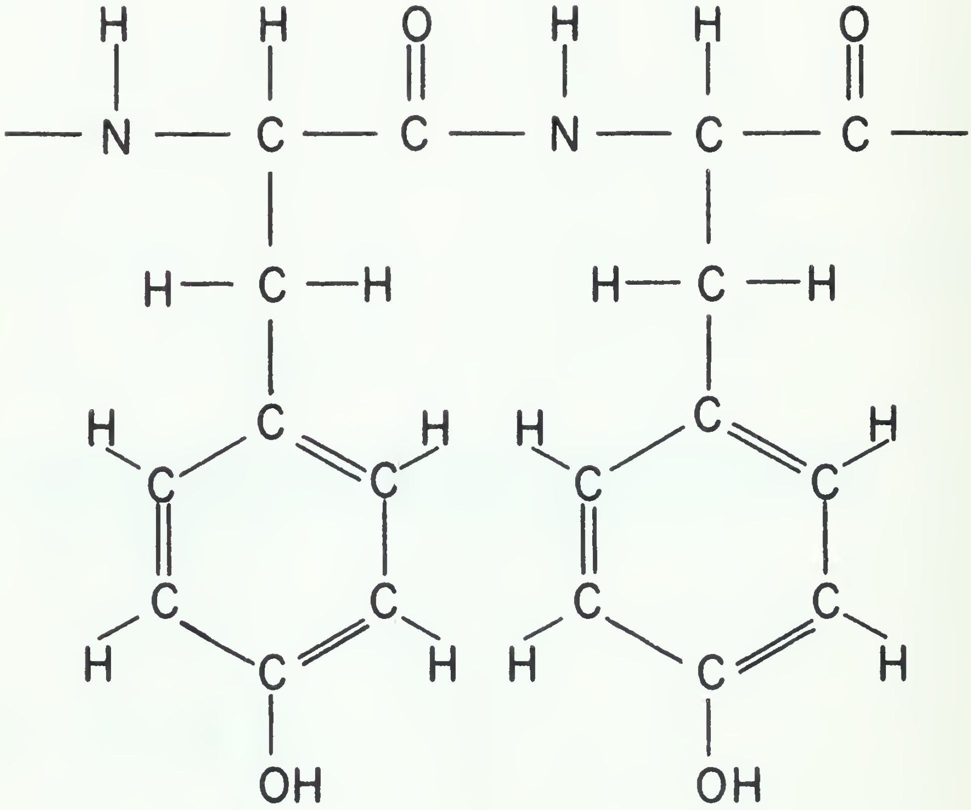
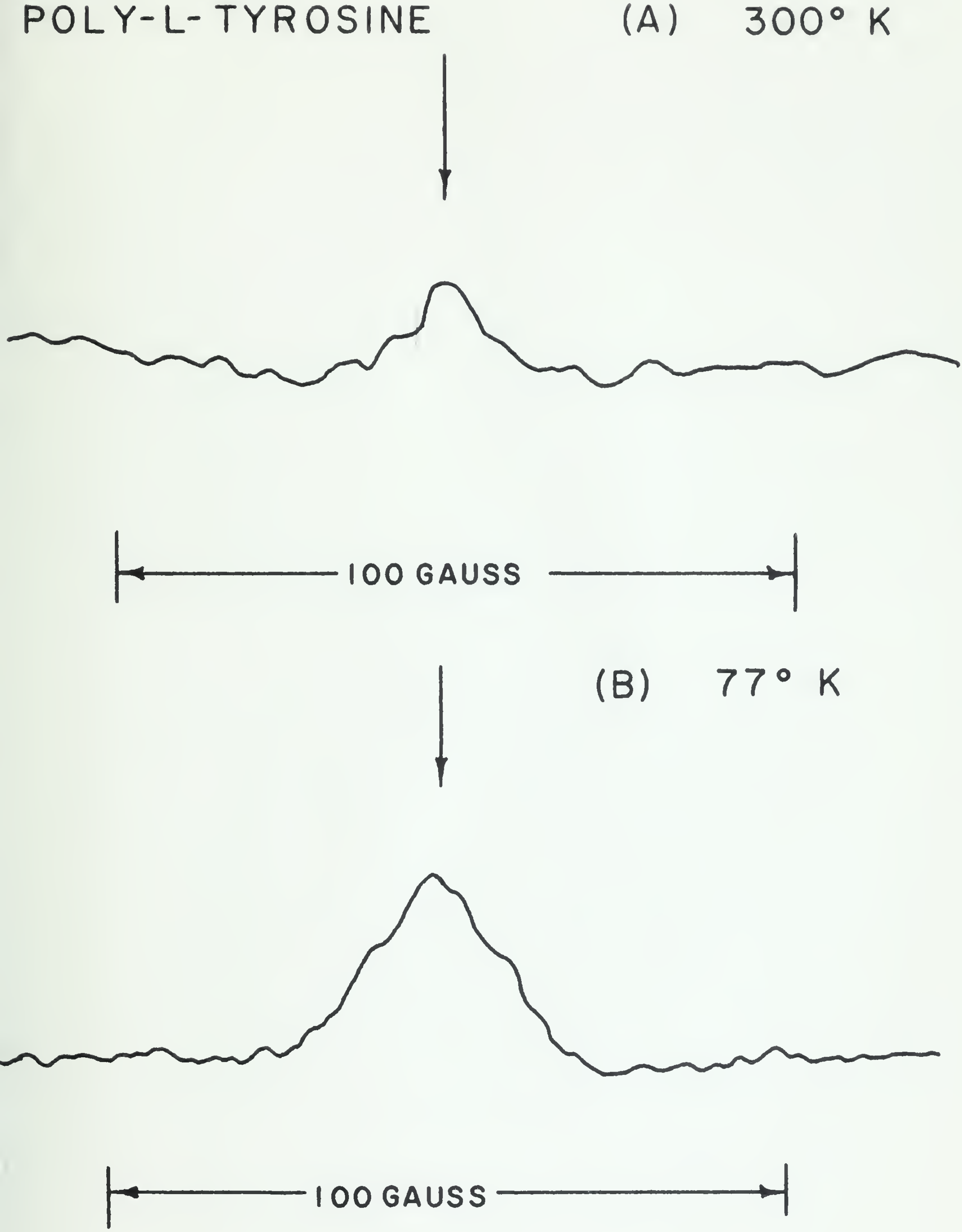


Fig. 25 (Above) Segment of a Poly-L-Tyrosine molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Tyrosine: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)


POLY-L-TYROSINE
(A) 300° K
100 GAUSS
(B) 77° K
100 GAUSS

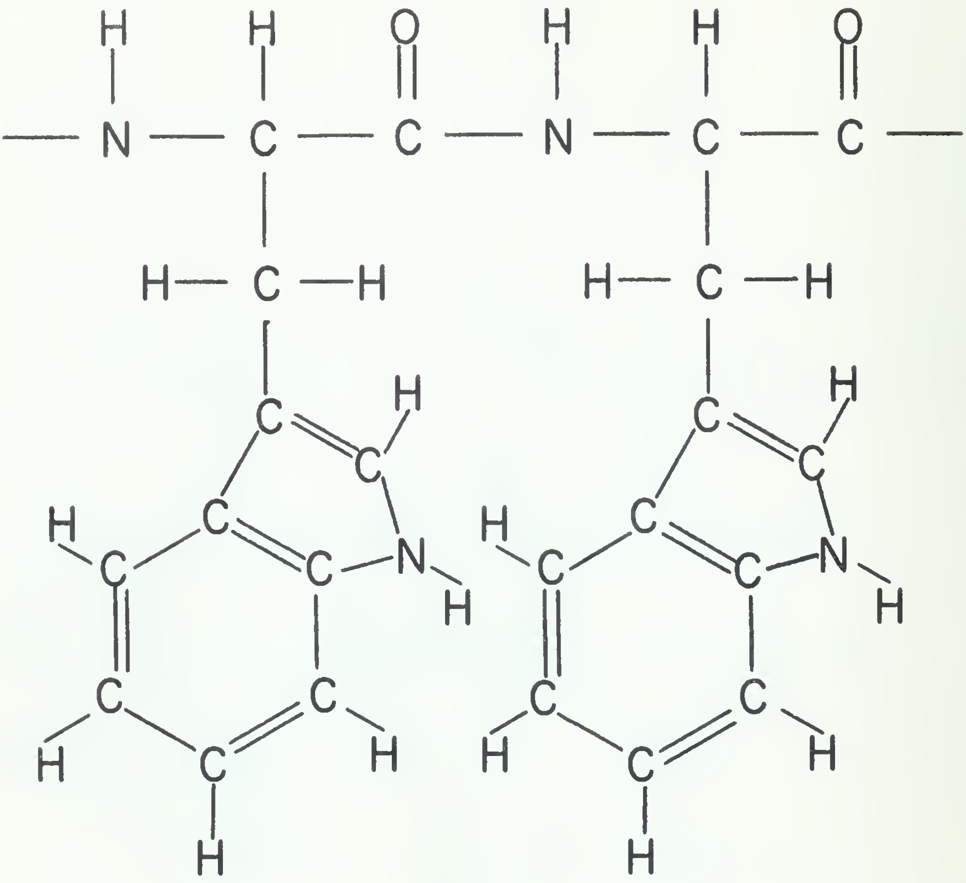
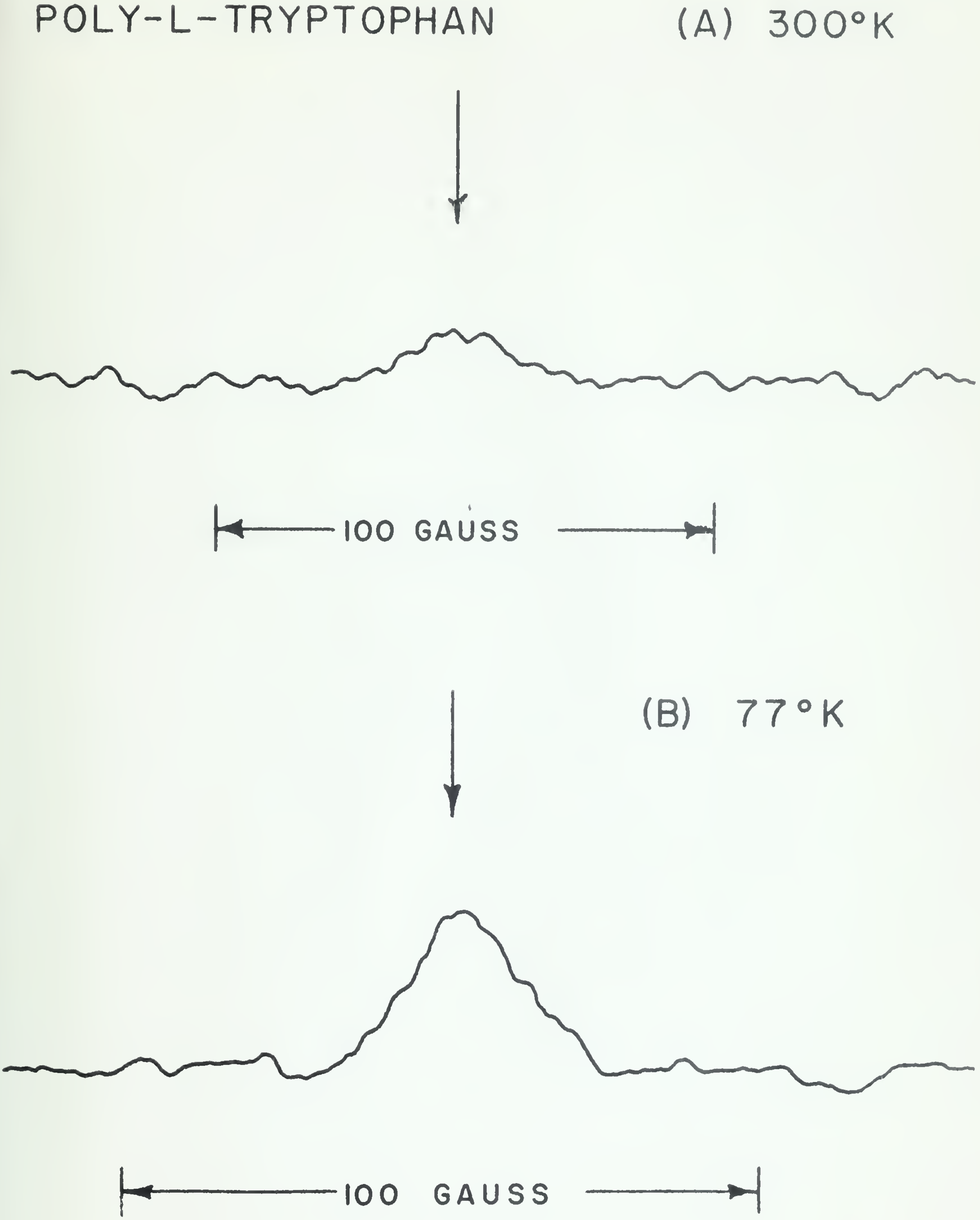


Fig.26 (Above) Segment of a Poly-L-Tryptophan molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Tryptophan: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)


POLY-L-TRYPTOPHAN
(A) 300°K
100 GAUSS
(B) 77°K
100 GAUSS

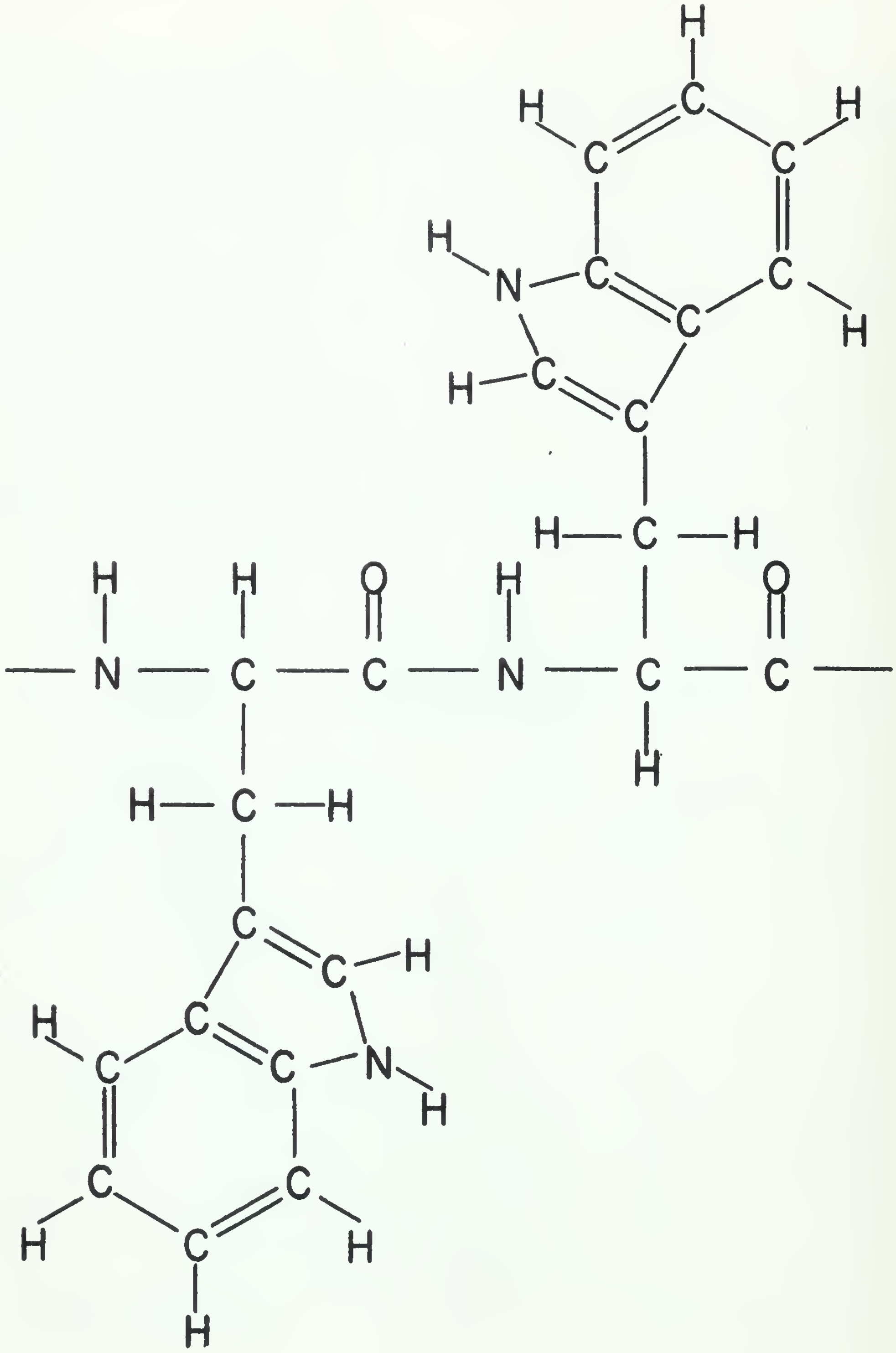


Fig. 27. (Above) Segment of a Poly-DL-Tryptophan molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-DL-Tryptophan: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

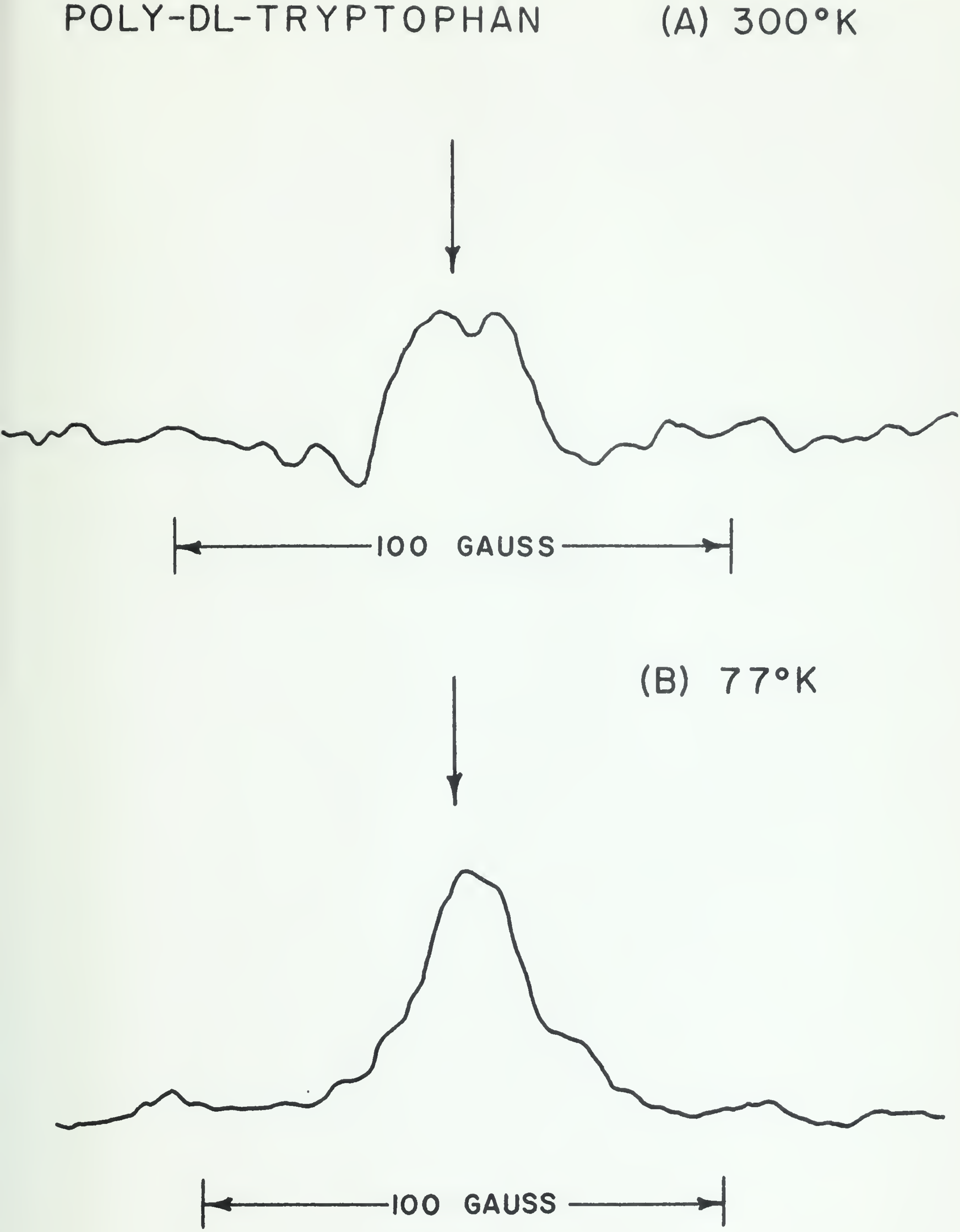
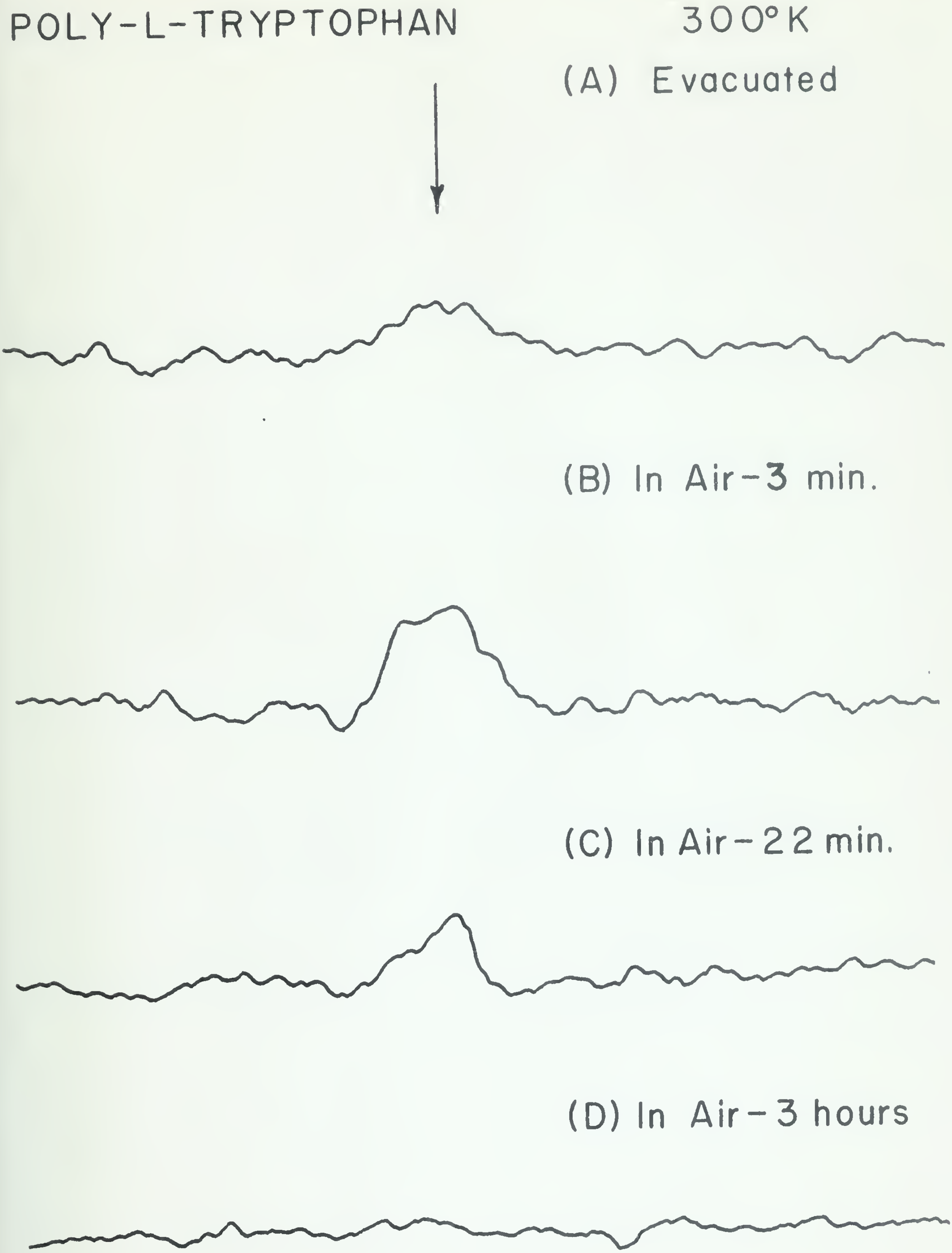

POLY-DL-TRYPTOPHAN
(A) 300°K
100 GAUSS
(B) 77°K
100 GAUSS

FIGURE 28. OPEN TO AIR SEQUENCE: POLY-L-TRYPTOPHAN (B) shows apparent increase in signal intensity immediately after being opened. (C) and (D) show subsequent decay of resonance. Spectrometer gain is the same (A)-(D).


POLY-L-TRYPTOPHAN
300°K
(A) Evacuated
(B) In Air-3 min.
(C) In Air-22 min.
(D) In Air-3 hours

end groups are expected to have no effect on the resonances.

Poly-L-aspartic acid at 300°K gives a triplet spectrum with intensities varying roughly as 1:2:1 and with an average splitting of 21 gauss between components. An unpaired electron left at the site of the α-carbon from removal of a single proton would interact with the protons of the adjoining carbon to give a spectrum of this nature. Removal of the proton from the OH group could also give a similar spectrum, providing the unpaired spin is delocalized sufficiently to interact with the adjoining CH_2 group.

When the sample is opened to air, the spectrum disappears within 4 minutes, leaving a weak singlet which then decays and becomes masked in the noise level. Poly-L-glutamic acid, with the addition of an additional CH_2 group to the same R side chain, gives a compound doublet when irradiated at 300°K which remains unchanged when exposed to air except for a gradual decrease in signal strength. The splitting between the lines is $\sim$21 gauss, while each primary line seems composed of two components separated by 3 gauss. The existence of a single radical in this molecule that would give this pattern is unlikely, and no attempt will be made to establish probable forms for multiple radicals in this case.

Anisotropic broadening caused by hindered rotation appears in the spectra for both samples when irradiated and observed at 77°K. For poly-L-aspartic acid, the central three components seem unchanged except for this broadening, while a weak pair of outer lines has appeared. In addition, the warming cycle appears to be reversible, which bears out this assumption.

For poly-L-glutamic acid, a set of four small outer lines appear, symmetrically positioned about the broad central resonance, which again seems to display a doublet character. When the sample is warmed (see Figure 31), the resonance gradually changes to a pattern different from that observed at

[illegible]

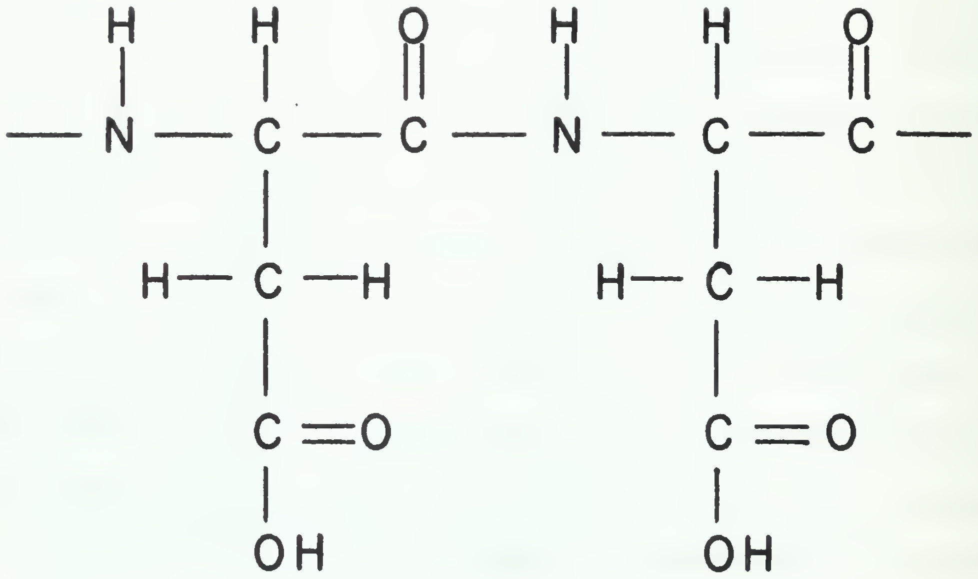
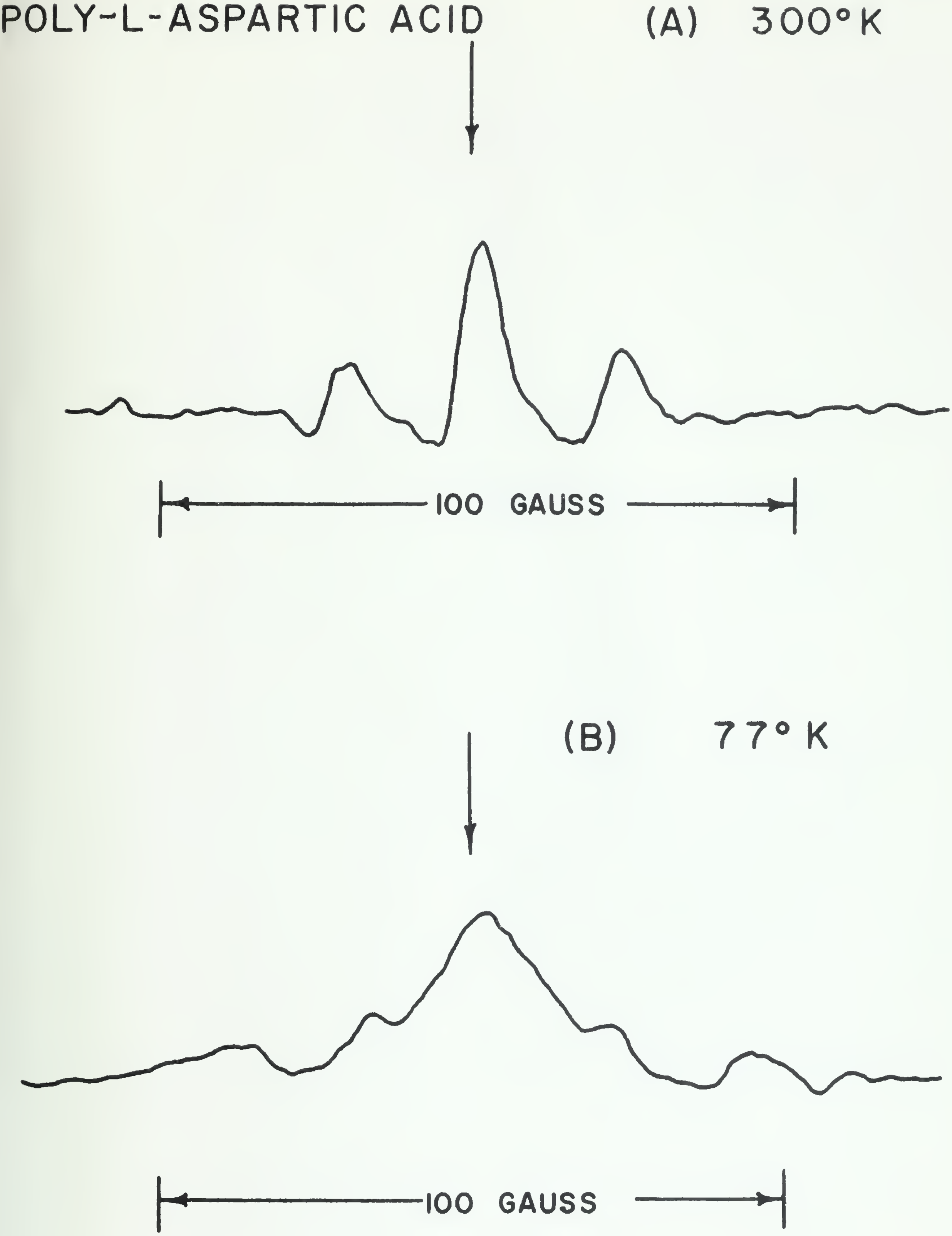


Fig. 29 (Above) Segment of a Poly-L-Aspartic Acid molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Aspartic Acid: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

POLY-L-ASPARTIC ACID



[illegible]

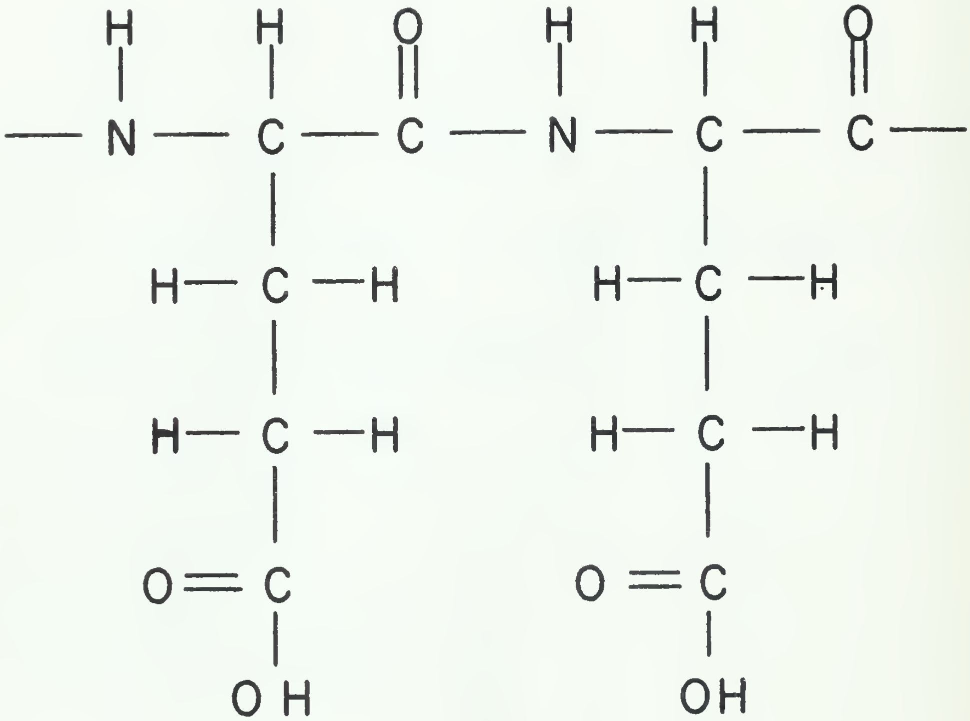
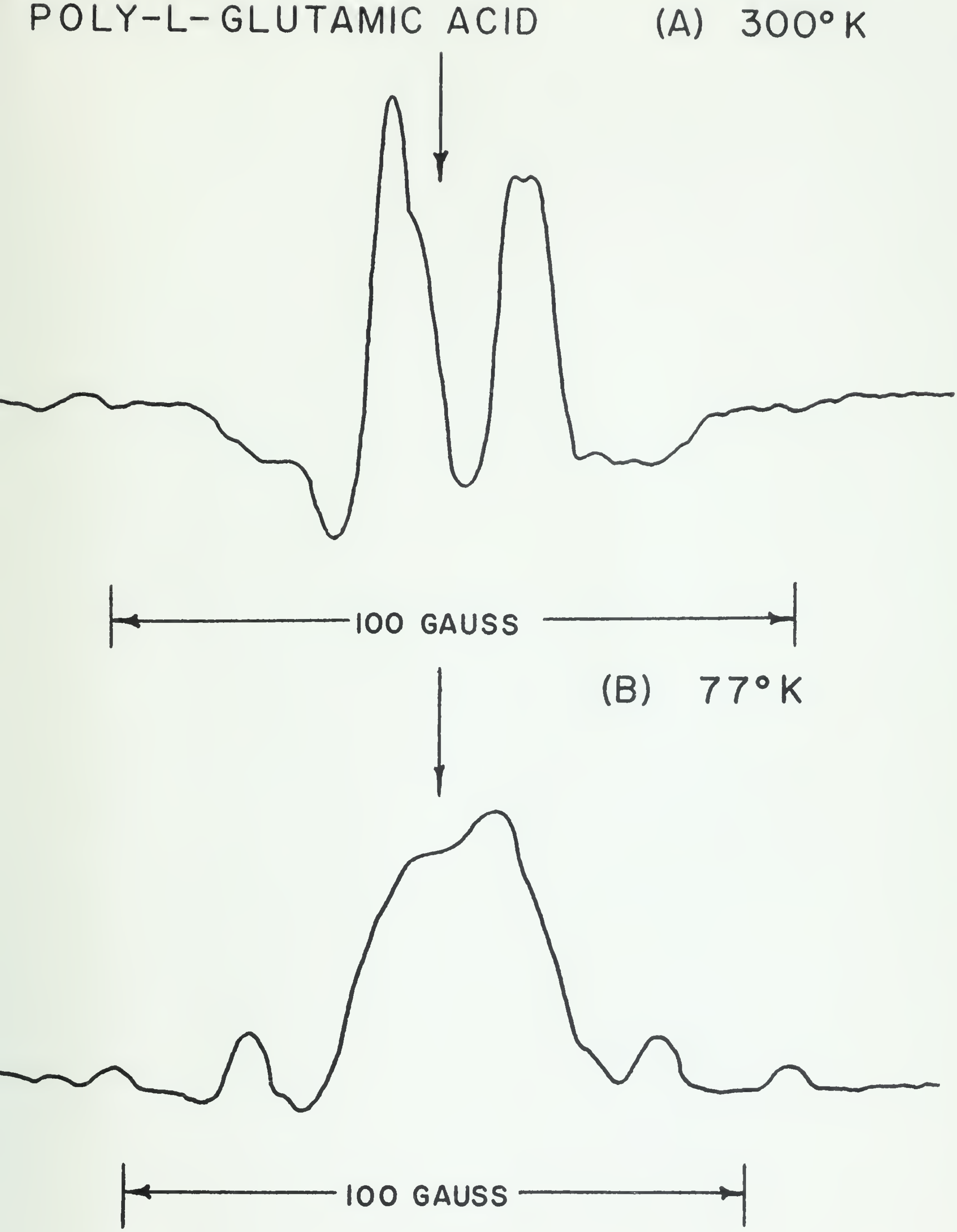
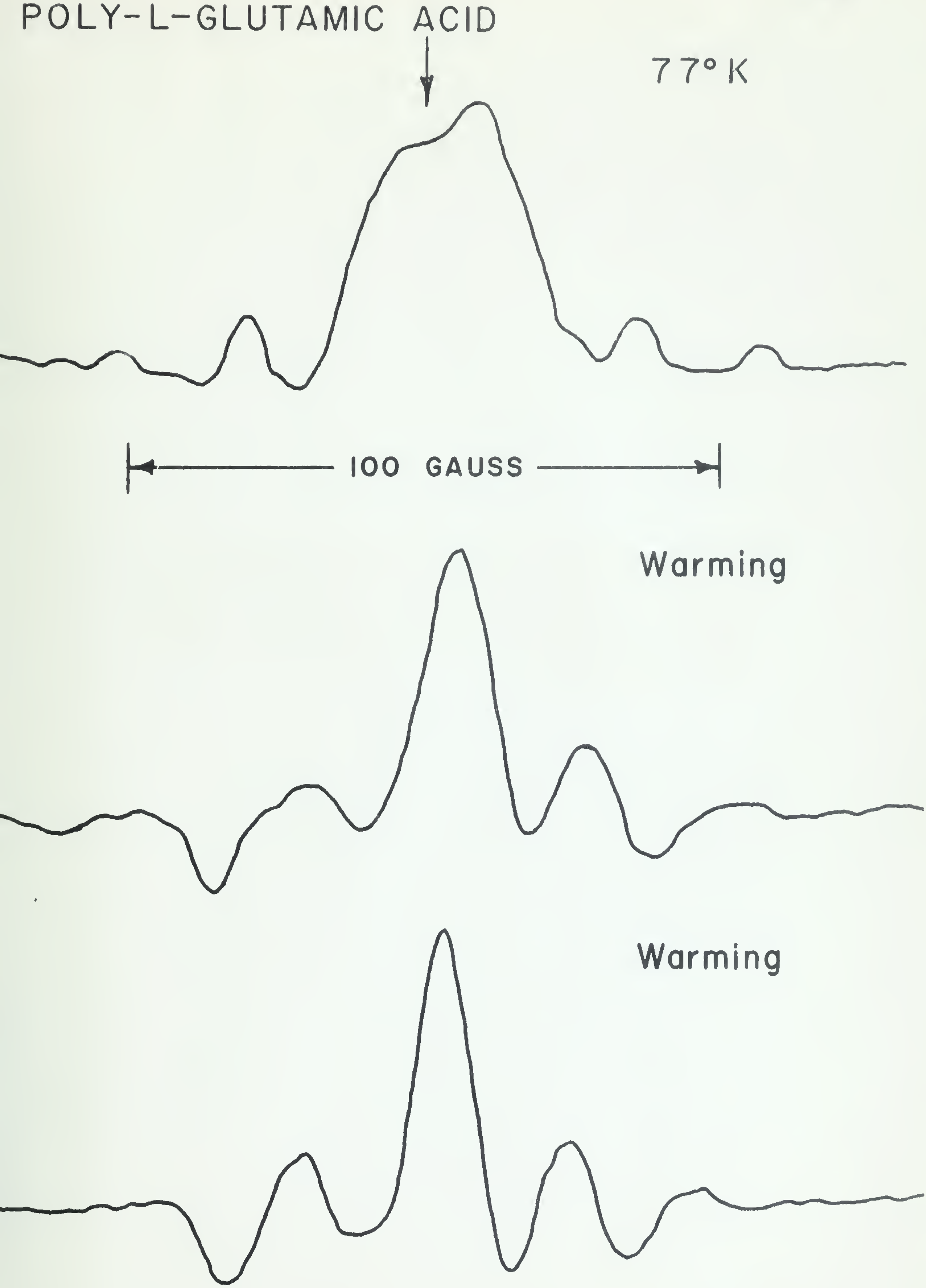


Fig. 30 (Above) Segment of a Poly-L-Glutamic Acid molecule. (Facing Page) Electron spin resonance curves for an evacuated sample of Poly-L-Glutamic Acid: (A) Irradiated and observed at 300°K. (B) Irradiated and observed at 77°K. (Second derivative representation.)

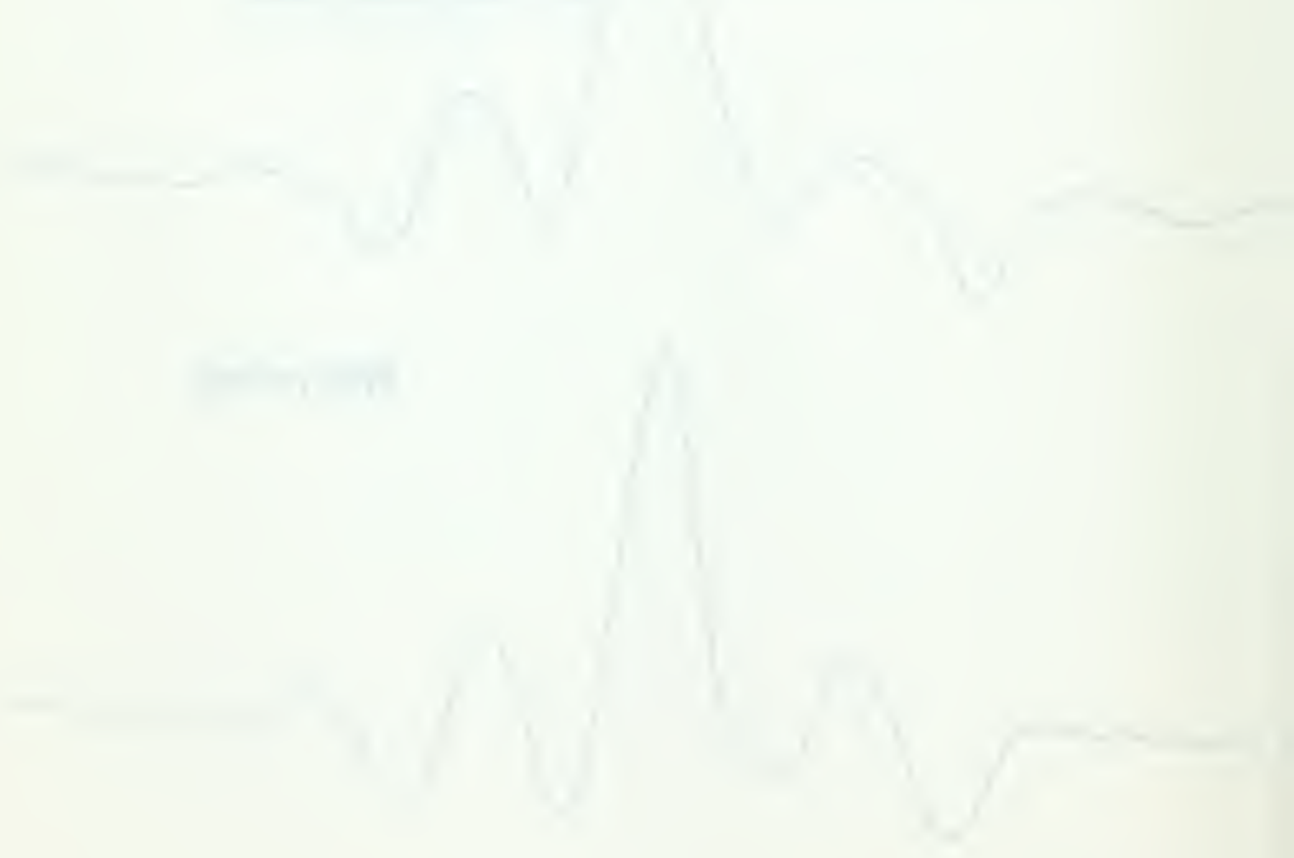

POLY-L-GLUTAMIC ACID
(A) 300° K
100 GAUSS
(B) 77° K
100 GAUSS

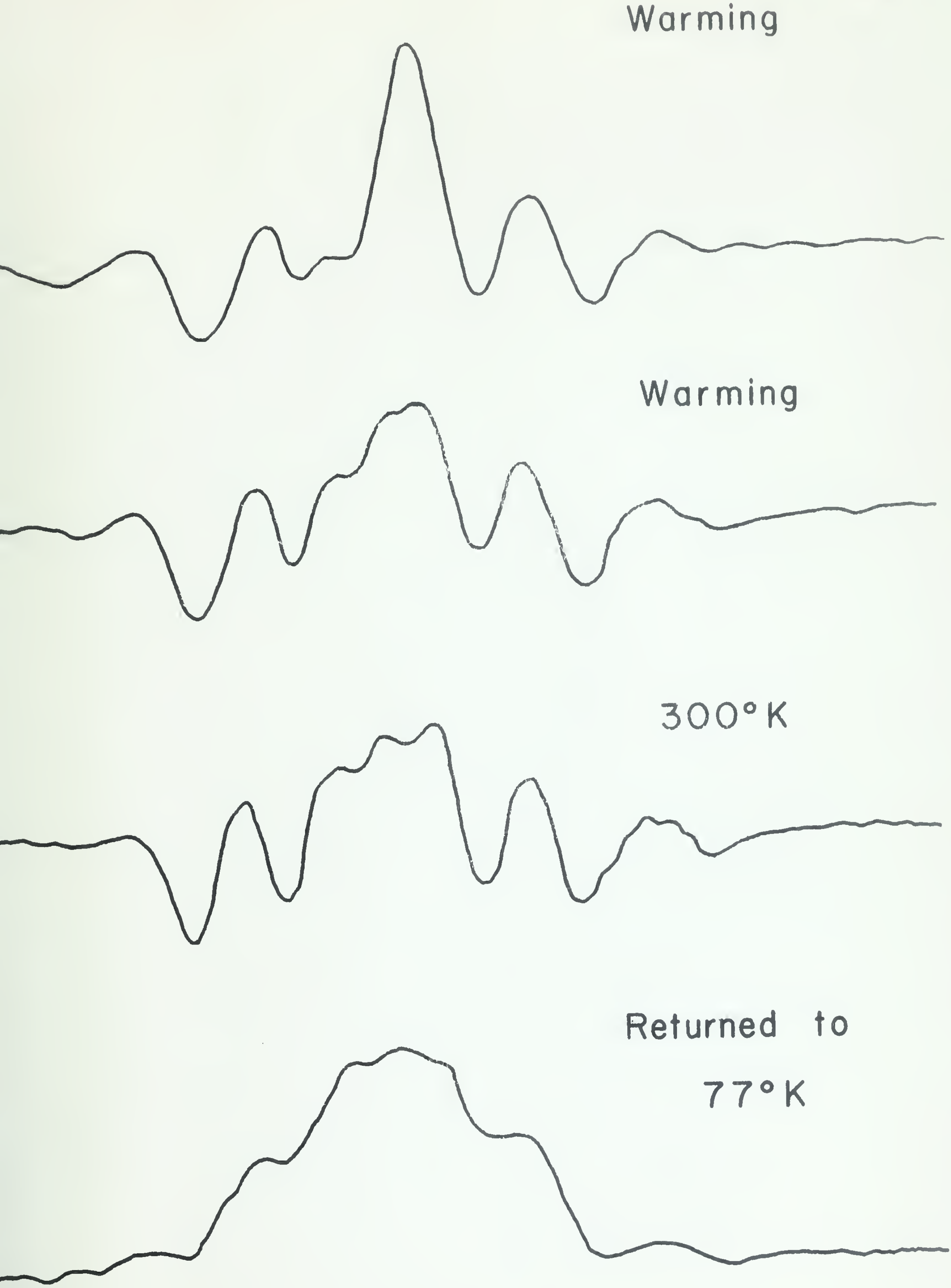
[illegible]

FIGURE 31. WARMING SEQUENCE: POLY-L-GLUTAMIC ACID (next two pages). Notice that the radical formed when irradiated at 77°K and then warmed differs from the radical or radicals formed when irradiated at 300°K. This is generally not true for the synthetic polypeptides investigated here.


POLY-L-GLUTAMIC ACID
77° K
100 GAUSS
Warming
Warming

[illegible]




Warming
Warming
300°K
Returned to
77°K

300°K. Here it appears that the radical or radicals formed at 77°K are of a different type from those formed at 300°K, an effect not observed in any other polypeptides except poly-L-phenylalanine, poly-DL-phenylalanine, and poly-O.acetyl-hydroxy-L-proline.

H. Poly-L-Lysine Hydrobromide. This compound is formed with the aid of anhydrous hydrogen bromide, HBr[53], leaving an ionic bond, as noted in the diagram of the molecule in Figure 31. This ionic character and the great length of the side group in this substance probably all contribute to the absence of a detectable resonance in an evacuated room temperature sample. At 77°K, however, molecular motions are retarded and recombination is inhibited sufficiently that a weak, broad, single resonance occurs with weaker side structure visible.

As the sample warms, anisotropic broadening is reduced, the migration of the spin density is facilitated, and in (B), the orbital of the unpaired electron has delocalized sufficiently to show interaction with 6 protons, giving the strong, sharp 7 line spectrum shown. This lasts only momentarily, however; and as illustrated in (C) and (D), a rather quick decay follows as greater molecular motions begin.

I. The Oxygen Effect. All the resonance patterns of the synthetic polypeptides observed thus far have displayed a rather strong effect upon the admission of air to an evacuated sample at 300°K. For some samples no change is observed other than an accelerated decay of the resonance. Others have their characteristic resonance replaced by a singlet, while still other resonances seem to be completely quenched. This could indicate that three different effects are taking place. It is proposed here, however, that the variation in behavior noted is solely one of degree rather than an indication that different mechanisms have been operating on the radicals formed.

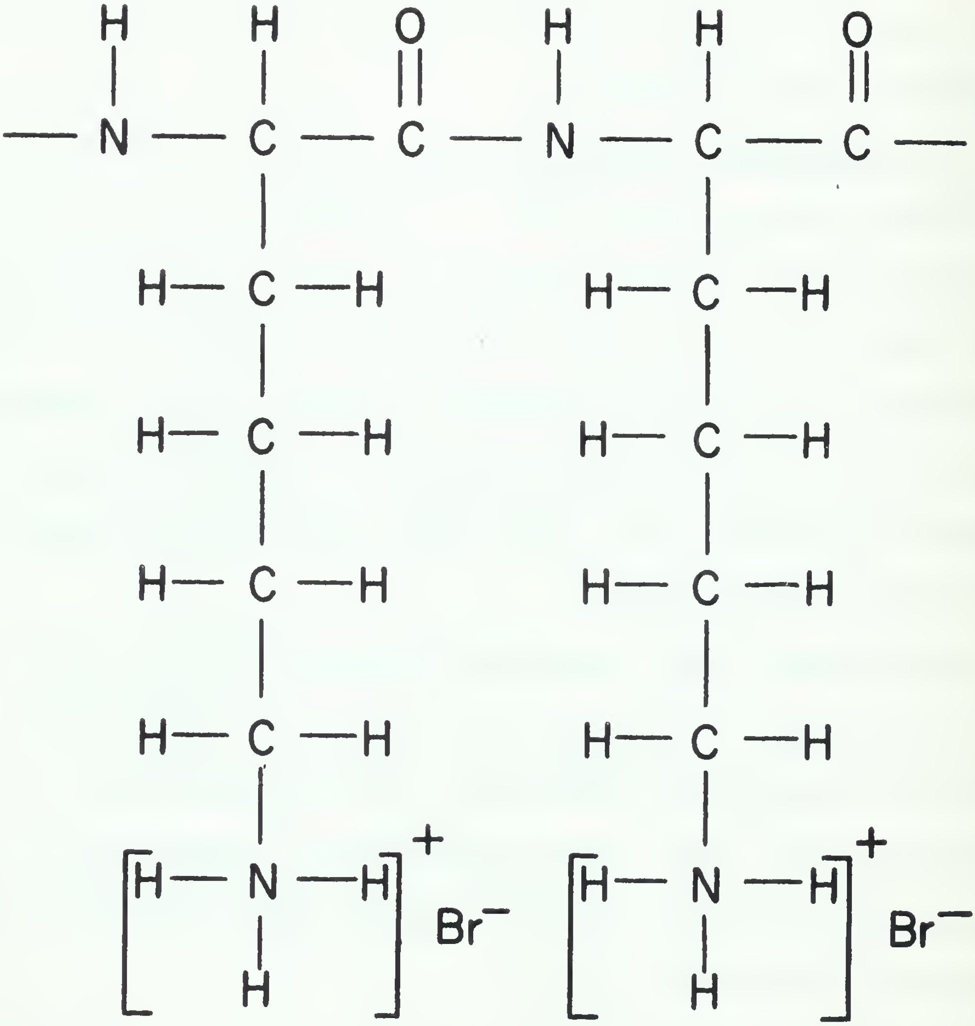
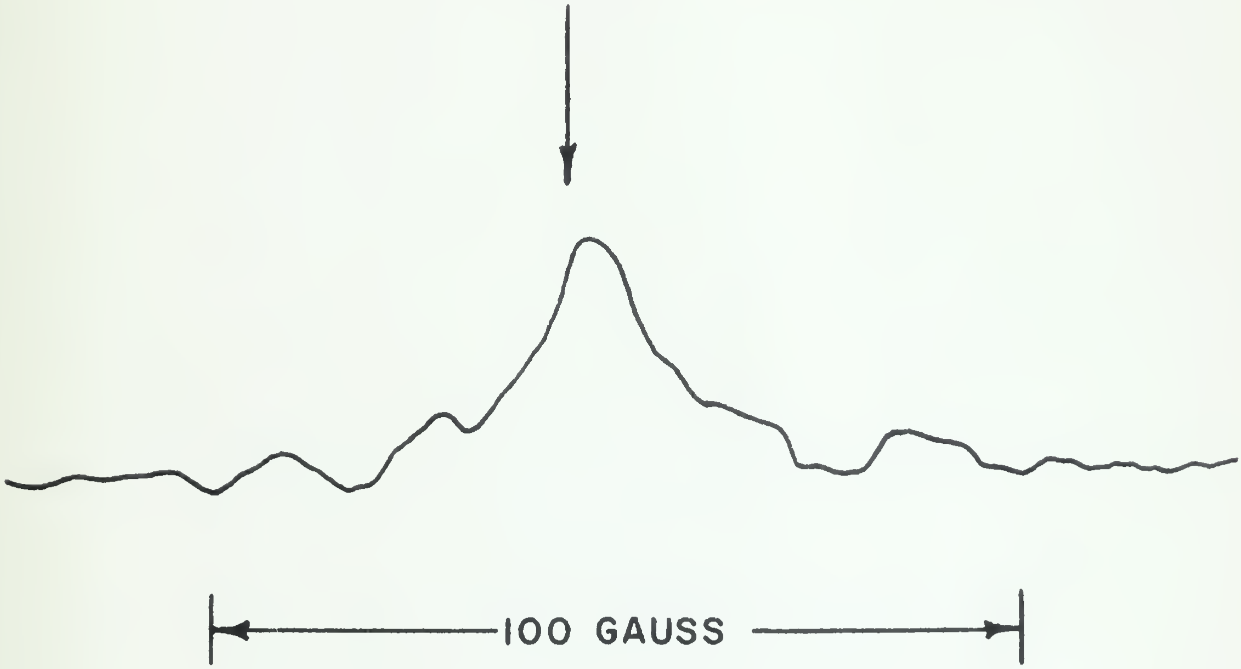


Fig. 32 (Above) Segment of a Poly-L-Lysine Hydrobromide molecule (Facing Page) Electron spin resonance curve for an evacuate sample of Poly-L-Lysine Hydrobromide irradiated and observed at 77°K. No detectable resonance was observed in an evacuated sample irradiated and observed at room temperatur (Second derivative representation.)

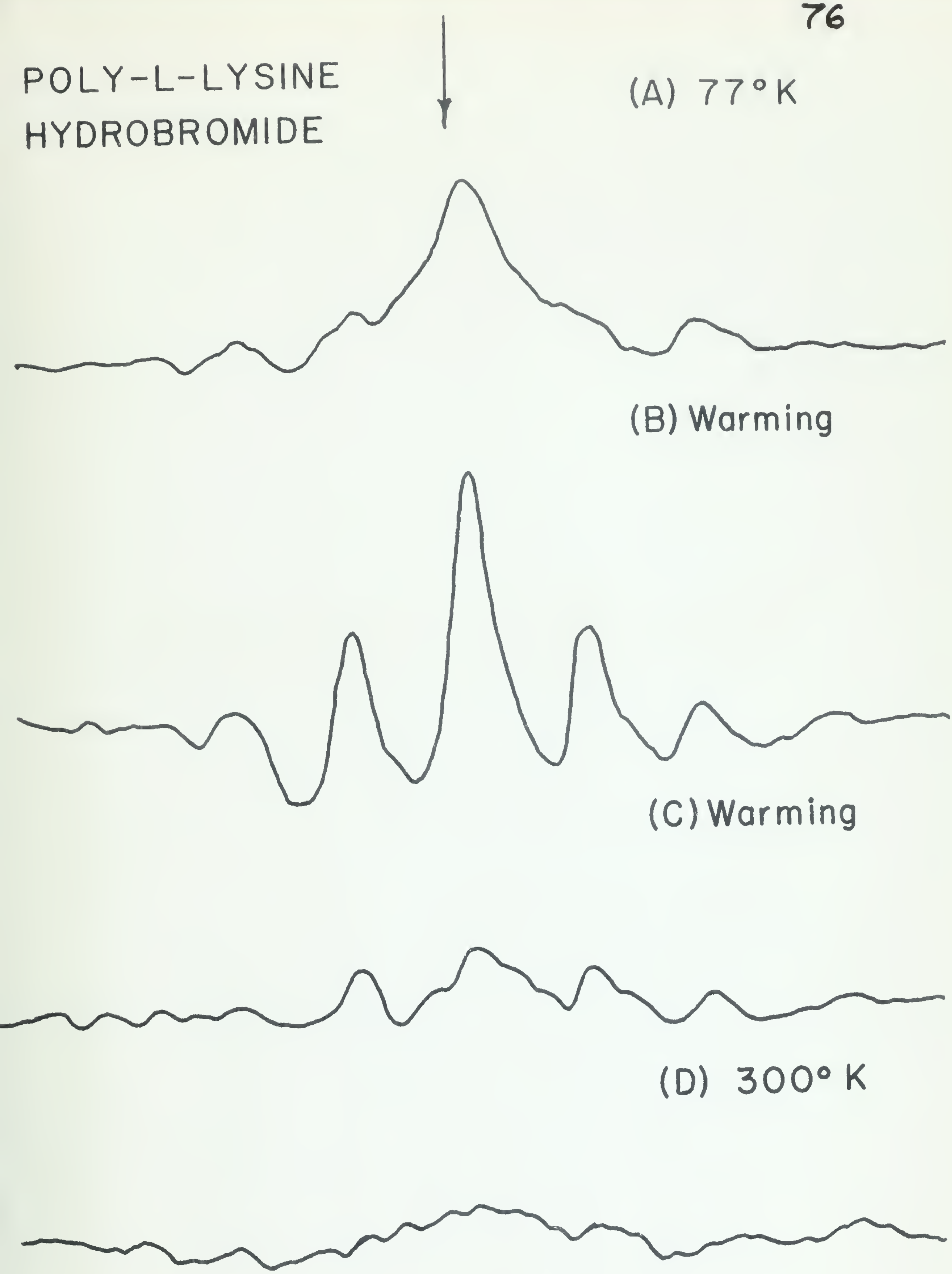
POLY-L-LYSINE HYDROBROMIDE

(A) 77°K



[illegible]

FIGURE 33. WARMING SEQUENCE: POLY-L-LYSINE HYDROBROMIDE
Note changes which occur ending in the quenching of the radical. Spectrometer gain is the same, (A)-(D).


POLY-L-LYSINE HYDROBROMIDE
(A) 77°K
(B) Warming
(C) Warming
(D) 300° K

Molecular oxygen has been shown[54, 55] to be the cause of similar effects observed in certain organic substances, and hence the term 'oxygen effect' was introduced. (NO has been found to cause a similar effect, usually termed an 'oxygen effect' by analogy.) In these polypeptide molecules, the mechanism is quite similar. Diffusion of molecular oxygen to the sites of the unpaired spins takes place and the electronegative oxygen combines with the previous radical to form a new peroxide radical, X-O-O•, where X is the previous radical. Here, the unpaired electron is localized in a three electron bond between the two oxygen atoms, and therefore no hyperfine structure would be expected. Furthermore, the flopping about of the O_2 axis at 300°K will reduce the spin-orbit coupling for this radical to make the resonance a fairly sharp singlet. More detailed investigation of the oxygen singlet reveals a field dependent asymmetry which is caused by anisotropy in the g-factor for the peroxide radical. The subsequent decay of the oxygen singlet would then be caused by reaction of the peroxide radical to form various non-radical products.

The three types of behavior for these polypeptides may then be explained with this one hypothesis. For poly-glycine, poly-sarcosine, poly-hydroxy-L-proline, and poly-L-glutamic acid, diffusion of oxygen to the radical sites is slow enough that decay of the peroxide radicals takes place at nearly the same rate, and concentration of the peroxide radicals is never large enough to be detectable. In this way, the only observable effect is the gradual decay of the initial resonance. The key rate determining mechanism here is the rate of diffusion of molecular oxygen to the spin sites.

In the second group, for which a singlet appears in place of the resonance, diffusion is sufficiently rapid to permit formation of the peroxide radicals, which then decay rather slowly. The decrease in signal intensity

here is now a measure of the decay rate of these peroxide radicals.

For the third group, for which the resonance is apparently completely quenched, the formation and decay of the peroxide radicals is sufficiently rapid that no detectable concentration accumulates. In this case, a high diffusion rate for oxygen and a high decay rate for the peroxide radical must combine.

From this discussion, it is evident that the spatial configuration can play an important role in this effect. A more open lattice or molecular structure would allow the diffusion of oxygen to the spin sites, while a tightly knit structure would restrict the attack by oxygen and maintain its characteristic spectrum over longer periods. Other factors may enter as well, i.e., the molecular oxygen is not likely to form a peroxide radical when the spin density is already concentrated on an oxygen atom, as in a case where the proton is removed from an OH group leaving O·.

A tabulation of the oxygen effect for the polypeptides investigated is included in Table I for convenient reference.

Table I

THE OXYGEN EFFECT FOR SYNTHETIC POLYPEPTIDES

1. 'Small' Effect—(spectrum retains the same character, but decays when opened to air)

    Poly-Glycine
    Poly-Sarcosine
    Poly-Hydroxy-L-Proline
    Poly-L-Glutamic Acid

2. 'Moderate' Effect—(spectrum changes to a singlet which then decays)

    Poly-L-Alanine
    Poly-DL-Alanine
    Poly-L-Leucine
    Poly-DL-Leucine
    Poly-L-Proline
    Poly-O.Acetyl-Hydroxy-L-Proline
    Poly-L-Tryptophan
    Poly-DL-Tryptophan

3. 'Strong' Effect—(spectrum is completely quenched before a sweep through the resonance can be made)

    Poly-L-Valine
    Poly-L-Phenylalanine
    Poly-DL-Phenylalanine
    Poly-L-Aspartic Acid

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Publications: "Electron Spin Resonance of γ-Irradiated Polypeptides," with Walter Gordy, Bull. Am. Phys. Soc., 6, 258 (1961).

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